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Guidance on the assessment of the safety of feed additives for the environment

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Abstract

This guidance document is intended to assist the applicant in the preparation and the presentation of an application, as foreseen in Article 7.6 of Regulation (EC) No 1831/2003, for the authorisation of additives used in animal nutrition. It specifically covers the assessment of the safety for the environment.

Keywords: guidance, environment, risk assessment, feed additives

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Main changes in the Guidance relative to the previous 2008 version

(This table will be deleted in the final version of the guidance document)

Topic	ERA Guidance 2008	Updated version
Specific protection goals	No mention	Following the EFSA Guidance on specific protection goals from the Scientific Committee, a description of the specific protection goals is available in Appendix A
Post marketing monitoring	No mention	Possibility that it is needed is considered in the introduction
Air compartment	No mention	Reasons for not dealing with it in the introduction
Decision tree of phase I	A three-step decision tree	There are some new questions with explanatory notes for each question. PBT and substances with a specific mode of action of concern or that accumulate in the environment go directly to phase II.
N load default value from manure application	The standard of the regulation: 170 kg N/ha per year	Although the previous N load is maintained, a rationale for a proposal to increase the nitrogen load to agricultural land from manure application from 170 to 250kg N/ha per year is available in Appendix G.
Phase I, Predicted environmental concentration (PEC) in soil and refinement based on degradation in manure	Default mixing depth of 5 or 20 cm in soil	Default soil incorporation depth of 5 cm assumed in PECsoil calculations also for poultry manure application, and PECsoil < 10 µg/kg dry weight, in Phase I.
Phase I, Table 1	Default values of feed intake and N excretion in the different species/categories	The values have been reviewed and in some cases updated. Some new species have been added. The assumptions and the different calculations are available in Appendix H.
PEC in sediment	Default 20 cm depth and < 10µg/kg wet weight	Default 5 cm depth, assuming 2±0.5% organic carbon (OC) and PECsoil <10µg/kg dry weight
Phase I, PEC in surface water, water flow rate in salmon farming	Default value of 1,400L/kg fish per day	Default value reduced to 865L/kg fish per day (data from Norwegian Food Safety Agency)
Phase II, introduction	Not described	General indications on how PEC and predicted no effect concentration (PNEC) estimates should be compared.
Phase IIA, PNECsoil	Plants test based on three plant species	Based on six different plant species.
Phase IIA, PECgw	Refinement based on advanced model only	Refinement of PECgw calculation based on experimentally derived Koc value in Phase IIA
Phase IIA, PNECsw and PNECsed	Sediment-water chironomid toxicity test for sediment	Trigger value for sediment effect assessment is log Koc or log Kow ≥3. PNECsed derived on basis of phase IIA PNECsw and Equilibrium partitioning concept

Phase IIA, PNEC marine compartment	No internationally recognised ISO or OECD guidelines were available	More detailed description of the studies to be performed. PNEC can be derived from sediment-spiked 10d toxicity tests with benthic organisms for which test protocols are available by applying an appropriate AF. In Phase IIC, chronic tests with these species will be considered.
Phase IIA, secondary poisoning	Log Kow ≥3 trigger for Bioaccumulation factor study (OECD 305)	In addition, quantitative structure-activity relationships (QSAR) calculation of bioconcentration factor, and cross-reference to ECHA guidance and VICH guidelines.
Persistent bioaccumulative and toxic substances	Not considered	Screening process and subsequent assessment proposed following EMA guidelines.
Phase IIC, PNECsoil refined, earthworm test	AF of 10 to the NOEC value of the earthworm test	An additional soil invertebrate test is needed (e.g. springtail OECD 232, or *Hypoaspis aculeifer* OECD 226). AF of 10 to the lowest EC_{10}/NOEC value. If there are still safety concerns, possibility to use the species sensitivity distribution (SSD) approach, semi-field tests or advanced modelling approaches (TK-TD and population models)
Phase IIC, PNEC fresh water including PNEC sediment		Indications for both pelagic and benthic organisms provided. Change of the assessment factor from 10 to 100 for NOEC or EC_{10} in case only one long term toxicity test is provided. The RHO solid changed from 1,300 to 2,500 kg/m^3. If there are still safety concerns, possibility to use the species sensitivity distribution (SSD) approach, semi-field tests or advanced modelling approaches (TK-TD and population models)
Phase IIC, PNECsed marine aquaculture	Acute toxicity tests with AF of 1000 or long term toxicity test with AF of 10 to NOEC.	Indications on available tests with benthic invertebrates and minimum requirements to derive PNECsed Change of the assessment factor from 10 to 100 for NOEC or EC_{10} in case only one long term toxicity test is provided If there are still safety concerns, possibility to use the species sensitivity distribution (SSD) approach, semi-field tests or advanced modelling approaches (TK-TD and population models)
Dung fauna	Not considered	Considered but not assessed due to the incapacity to identify dung fauna to protect in chicken manure.
Appendix B, Application of FOCUS models in ground water. Section 6 Application.	Default value for depth (m) was 0.2 (realistic worst case)	Default values for depth (m) are now 0.05 for $PEC_{soil;tot}$ 0.2 for $PEC_{soil;pw}$ (realistic worst case)

Appendix D, Data requirements and quantitative structure-activity relationships calculations	Not considered	Screening approach to apply in a case by case basis for feed additives.
Assessment of risk to groundwater	In Phase I, $PEC_{ground\ water}$ is estimated and compared with the threshold value. Although in Phase II $PEC_{ground\ water}$ is refined, no acceptable levels are determined.	The groundwater quality standard for pesticides as reported in the groundwater directive is considered as the reference value for the evaluation.

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Background and Terms of Reference

Regulation (EC) No 1831/2003 establishes the rules governing the Community authorisation of additives for use in animal nutrition. Moreover, Regulation (EC) No 429/2008 provides detailed rules for the implementation of Regulation (EC) No 1831/2003 as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives.

The Panel on Additives and Products or Substances used in Animal Feed (FEEDAP Panel) has adopted a series of guidance documents which aim at complementing Regulation (EC) No 429/2008 to support applicants in the preparation and submission of technical dossiers for the authorisation of additives for use in animal nutrition according to Regulation (EC) No 1831/2003.

The European Food Safety Authority (EFSA) asked its FEEDAP Panel to:

1. identify from the current guidance documents, those that need to be updated, taking into consideration the most recent scientific developments and the experience gained in the assessment of feed additives;
2. update the guidance documents in need of revision accordingly; this activity can be conducted in different rounds on the basis of the priorities identified and on the feasibility of the revision according the resources available;
3. taking into account the sensitivity and the relevance of some of the guidance documents under revision and the entity of the revision itself (e.g. substantial or not), consider initiatives like preparatory info-sessions or public consultations of the draft guidance documents. The relevant comments received in either step will have to be considered and addressed if appropriate in the final version of the guidance documents.

The first of the terms of reference was addressed by a statement of the FEEDAP Panel (EFSA FEEDAP Panel, 2016), in which it was identified the need to update most of the guidance documents that it produced and set priorities for this update.

This output addresses the second and third terms of reference with regards to the update of the guidance documents dealing with the assessment of the environmental risk of feed additives.

Scope of the guidance

This guidance document is intended to assist the applicant in the preparation and the presentation of its application, as foreseen in Article 7.6 of Regulation (EC) No 1831/2003. This document does not substitute for the obligation of an applicant to comply with the requirements of Regulation (EC) No 1831/2003 and its implementing rules. This guidance document is intended to provide the information necessary to properly assess the environmental impact of a feed additive, in order to demonstrate compliance with the requirements of Article 5.3 of Regulation (EC) No 1831/2003.

Applicants should justify the omission from the dossier of any data or any deviations from the requirements detailed in this guidance.

A feed additive may be a well characterised chemical or agent (e.g. a crystallised amino acid of >98% active substance); a mixture of active chemicals or agents each of which is clearly definable (qualitatively and quantitatively); or a complex mixture in which not all constituents can be identified (typically plant extracts, containing several different chemically defined and/or undefined compounds). Different risk assessment procedures are considered. When the additive contains one or more clearly definable chemicals or agents, the ERA described in this guidance should be performed for each chemical/agent.

For complex mixtures with unidentified constituents, the FEEDAP Panel notes that developing an environmental risk assessment for such mixtures is not in the scope of the present guidance. The EFSA Scientific Committee is currently developing a guidance to assess mixtures of chemicals. Once the Scientific Committee of EFSA has officially published their guidance on risk assessment for mixtures, the FEEDAP Panel will consider it in a future update of this guidance.

When assessing the impact of microorganisms used as feed additives to the environment, the following scenarios may apply:

- For microorganisms included in the QPS list, any impact on the environment is assessed in the framework of the qualified presumption of safety (QPS) evaluation (EFS BIOHAZ Panel, 2017). When the identity of such a microorganism included in the QPS list is unequivocally established and any qualification (if existing) is met, safety for the environment is presumed.
- Strains carrying acquired genes for antimicrobial resistance are presumed to pose a risk for human and animal health via the environment.
- For microorganisms not included in the QPS list the following applies:

  - For those naturally present in soils, plants or gastrointestinal tract of animals, their use as feed additive is considered unlikely to measurably increase numbers in the environment where they are already prevalent. Consequently, the Panel considers that their use as feed additives would not pose a risk for the environment.

  - For those not naturally present in soils, plants or gastrointestinal tract of the animals, a case-by-case assessment would be needed. The principles of an OECD Guidance to the environmental safety evaluation of microbial biocontrol agents (SANCO/12117/2012 –rev. 0) may be used as a guide. Furthermore, the European Commission is currently developing a guidance document on the risk assessment of metabolites produced by micro-organism after application as active substances in plant protection products. Such guidance document can be considered in a future update of this guidance.

This guidance is divided in four sections. The introduction provides the principles of the environmental risk assessment (ERA) for feed additives. A Phase I decision tree is provided in Section 2, including the predicted environmental concentrations (PECs) for feed additives for terrestrial and aquatic environments. The PEC formulas and related default values were derived from the European Medicines Agency (EMA) guidance for the environmental risk assessment of veterinary medical products. The Phase II assessment, containing information on determination of predicted no effect concentrations (PNECs), on refinement of PECs, and refinement of PNECs is given in Section 3. Section 3 includes also the assessment of persistent, bio-accumulative and toxic (PBT) substances and the assessment for secondary poisoning. Section 4 describes how to provide information on studies retrieved from the literature.

1. Introduction

This document provides guidance on how to conduct and report studies concerning the assessment of the safety of feed additives for the environment. It is an update of the previous one (EFSA, 2008a) and supersedes it.

Consideration of the environmental impact of feed additives is important since administration of these substances typically occurs over long periods, often involves large groups of livestock animals and the constitutive active substance(s) may be excreted to a considerable extent either as the parent compound or its metabolites.

Regulation (EC) No 1831/2003 and its implementing rules (Regulation (EC) No 429/2008) describe that an environmental risk assessment (ERA) should be conducted for (1) terrestrial compartment (via spreading of animal manure contaminated with feed additives on agricultural soils), (2) the aquatic compartment (via drainage and run-off from agricultural fields to surface water, via direct discharge of waste water from land-based fish farms to surface water, or via excreta from fish farmed in cages to sediment), and (3) the groundwater compartment (via leaching from soil). As referring to the air compartment, according to ECHA (2008), "methods for the determination of effects of chemicals on species arising from atmospheric contamination have not yet been fully developed, except for inhalation studies with mammals. Therefore, the methodology used for hazard assessment (and therefore the risk characterisation) of chemicals in water and soil cannot be applied yet in the same manner to the atmosphere."

The ERA decision schemes described in this document aim to protect non-target plant and animal species in the receiving environment at the population level, while the protection level for microbes and protozoans is set at the biological functional group level.[1] As default the 'ecological threshold option' (see appendix A) is selected as specific protection goal (SPG). In this option the magnitude of tolerable effect on key organism groups in the receiving environment is set at small (e.g. <10% effect relative to controls). The ERA for feed additives (and their metabolites) is based on the precautionary principle meaning that, in the absence of relevant and reliable data, the PEC and PNEC estimates are based on worst-case assumptions, which could be refined by generating more relevant and reliable data.

To determine the environmental impact of feed additives, a stepwise approach is followed. All feed additives should be assessed through Phase I to identify those feed additives which do not need further testing. For the other feed additives a second phase (Phase II) assessment is needed. Additional information has to be provided, based upon which further studies may be considered necessary. Some feed additives that might otherwise stop in Phase I may require additional environmental information to address particular concerns associated with their potential risk. These situations are expected to be the exception rather than the rule and some evidence in support of the concern should be available.

The option of post marketing monitoring should be considered in the case that the negative effects of feed additive on the environment could not be undoubtedly excluded.

For the purpose of this guidance, the following definitions apply:

- Active substance: any substance or mixture of substances intended to be used as/in a feed additive that provides the intended effect.[2]
- Active agent: any microorganism intended to be used as/in a feed additive and that provides the intended effect.
- Feed additive: substances, microorganisms or preparations other than feed materials and premixtures which are intentionally added to feed or water in order to perform one or more functions mentioned in Article 5.2 of Regulation (EC) No 1831/2003.

2. Phase I assessment

The purpose of Phase I assessment is to determine if a significant environmental effect of the additive is likely and whether a Phase II assessment is necessary. Phase I is based on a list of exclusion criteria structured in a decision tree. By using a minimum set of information, it is aimed to screen additives that do not need a Phase II ERA. The ERA of major species can be extrapolated to minor species when the same use is proposed.

Exemption from Phase II assessment may be made on the following criteria, unless there is scientifically-based evidence for concern:

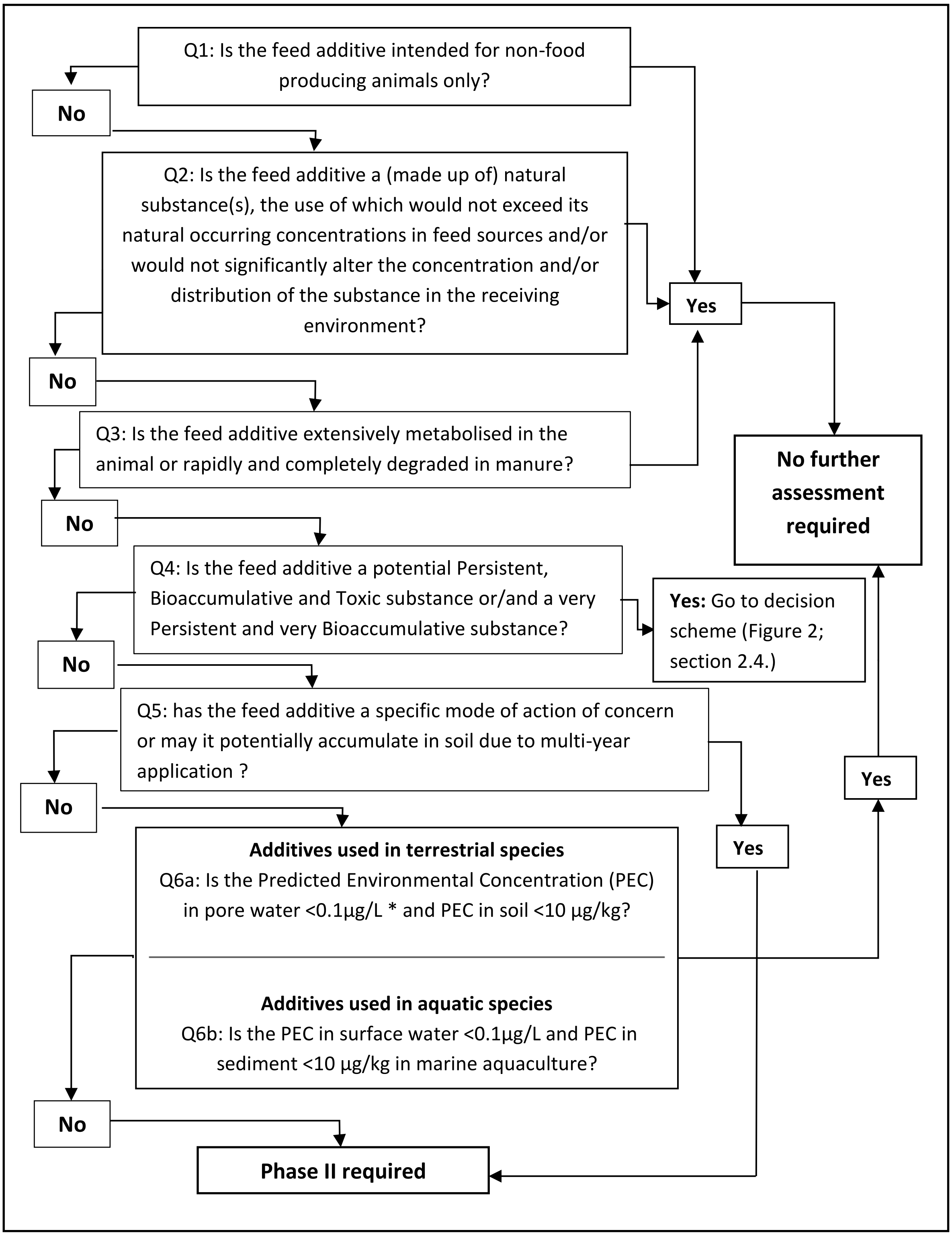
- The additive is intended for non-food producing animals only;
- The additive is a natural substance, or made of natural substances, the use of which as a feed additive would not exceed its natural occurring concentrations in feed sources, and/or would not substantially alter the concentration and/or distribution of the substance in the receiving environment;
- The additive is extensively metabolised in the target animal;
- The feed additive is not a potential persistent, bioaccumulative and toxic (PBT) or/and very persistent and very bioaccumulative (vPvB) substance;
- The additive does not trigger concern due to a specific mode of action or due to accumulation in the receiving environment over the years; and

[1] According to the "Guidance to develop specific protection goals options for the environmental risk assessment at EFSA, in relation to biodiversity and ecosystem services" (EFSA Journal 2016:14(6):4499) a functional group is a collection of organisms with similar functional trait attributes and that are likely to be similar in their response to environmental changes and effects on ecosystem functioning.

[2] Mixtures of substances means mixture of chemicals and/or agents

- The PEC for each compartment of concern, calculated based on (i) the annual input of the manure, (ii) the assumption that 100% of the dose ingested is excreted as the parent substance, does not meet the threshold value that triggers a Phase II assessment.

A decision tree is presented below (see Figure 1: Quick check), with explanatory notes for each question in sections 2.1 – 2.7.



* PEC in ground water is set equal to PEC in pore water (see Section 2.6.2)

Figure 1: Quick-check – Environmental Risk Assessment: Phase I

Further clarifications on these questions are given in the following subsections

2.1. Question 1: Is the feed additive intended for non-food producing animals only?

Generally, non-food producing animals are not intensively reared and/or their excrements are not spread over agricultural land. Therefore, due to the limited total amount of product used, feed additives for non-food animals are expected to produce less environmental concern than the feed additives in food-producing animals. As a consequence, besides exceptional cases (e.g., additives used in intensively reared fur-producing animals), no further assessment is required (Figure 1). For those exceptional cases, the ERA would proceed through the following questions.

2.2. Question 2: Is the feed additive a (made up of) natural substance(s), the use of which would not exceed its natural occurring concentrations in feed sources and/or would not significantly alter the concentration and/or distribution of the substance in the receiving environment?

Evidence should be provided showing that comparable concentrations of the feed additive can be expected in other plant(s) and/or that the use of the feed additive will not significantly alter the concentration of the additive in the receiving environmental compartments of concern. For this purpose, the excretion rates (as active substance) in target species exposed to the additive at the highest permitted level in the EU or at the highest intended concentration in feed, should be compared with the lower ranges of reported background concentrations in soils, water and plants. If applicable, its degradability in the receiving environment may also be considered. Evidence on which to base such scientific rationale should be provided. This evidence can be based on available information retrieved from structured literature reviews (see Section 4) or on analytical data.

For instance, if the concentration of a colouring agent used in fish feed is similar to that encountered in the natural diet of the fish species of concern (see FEEDAP Panel, 2014), or the concentration of a flavouring compound in feed does not exceed its natural concentration in plants (see EFSA FEEDAP Panel, 2016), no adverse impact is expected for the environment.

2.3. Question 3: Is the feed additive extensively metabolised in the target animal or rapidly and completely degraded in manure?

A feed additive is considered to be "extensively metabolised" if converted into metabolites present in the excreta that do not possess a biological activity of environmental concern, like water, CO_2 and common salts. A similar approach as in EMA 2016 is followed: As a part of the Phase I assessment, data (analytical and/or from the scientific literature, see Section 4) on degradation of the active residue in manure may be submitted. If the active residue is rapidly and completely degraded in manure then the assessment may end at Phase I." In order to fully satisfy the requirements and to be in compliance with the definition of extensive metabolism, complete degradation should be demonstrated either by total mineralisation or by the presence of degradation products all representing ≤5% of the initial concentration in feed. When the application covers several target species/categories, it is recognised that it may be very demanding to provide studies for all potential target species receiving the feed additive. Therefore, inter-species extrapolation of data can be applied. The applicant is referred to the guidance on the assessment of the safety of feed additives for the consumer (Section 2.1.1.1) to select the most representative species to be investigated.[3]

[3] EFSA Journal 2017;15(10):5022

2.4. Question 4: Is the feed additive a potential Persistent, Bioaccumulative and Toxic substance or/and a very Persistent and very Bioaccumulative substance?

Substances that are persistent, bioaccumulative and toxic (PBT) or very persistent and very bioaccumulative (vPvB) are of very high concern (REACH Regulation (EC) No. 1907/2006).[4] Due to the combination of these intrinsic properties and possible redistribution across environmental compartments, they pose serious hazards to non-target organisms.

Substances are considered as PBT or vPvB substances when they fulfil the criteria as laid down in Annex XIII of the REACH Regulation (EC) No 1907/2006,[5] for all three inherent properties P, B and T or both of the inherent properties vP and vB, respectively. To ensure a harmonised approach, these criteria together with the methodology in the current REACH guidance on PBT-assessment (ECHA, 2017a,b,c,d) and the guideline on the assessment of persistent, bioaccumulative and toxic (PBT) or very persistent and very bioaccumulative (vPvB) substances in veterinary medicinal products (EMA, 2015), should be considered.

If based on the available information or screening information the active substance is a (potential) PBT and/or vPvB substance, a separate PBT/vPvB assessment in phase II needs to be conducted. Where only screening information is available for one or more endpoints, the first step consists in screening whether the substance may fulfil the criteria. Screening information listed in Appendix E can be used as a help for comparing the screening information with screening thresholds (screening criteria) established for this purpose (for further details, see ECHA Guidance Chapter 11 on PBT/vPvB assessment (ECHA, 2017a) and ECHA Guidance on information requirements and chemical safety assessment Part C (ECHA, 2017e), Section C.4.1). If for one or more endpoints the technical dossier contains only the information as required in Phase I, the applicant (based on screening information and other information available) must:

- either derive an unequivocal conclusion that the substance does not fulfil the criteria; or
- when this is not possible and there are indications that the substance may fulfil the criteria, the applicant must obtain further information needed to fulfil the objective of the PBT and vPvB assessment.

The applicant should explain why the models they have used are appropriate for the substance in question.

A decision scheme for assessing PBT or vPvB properties of the feed additive is presented in Figure 2.

[4] Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) and establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC. OJ L 396, 30.12.2006, p. 1

[5] OJ L 396, 30.12.2006, p. 1

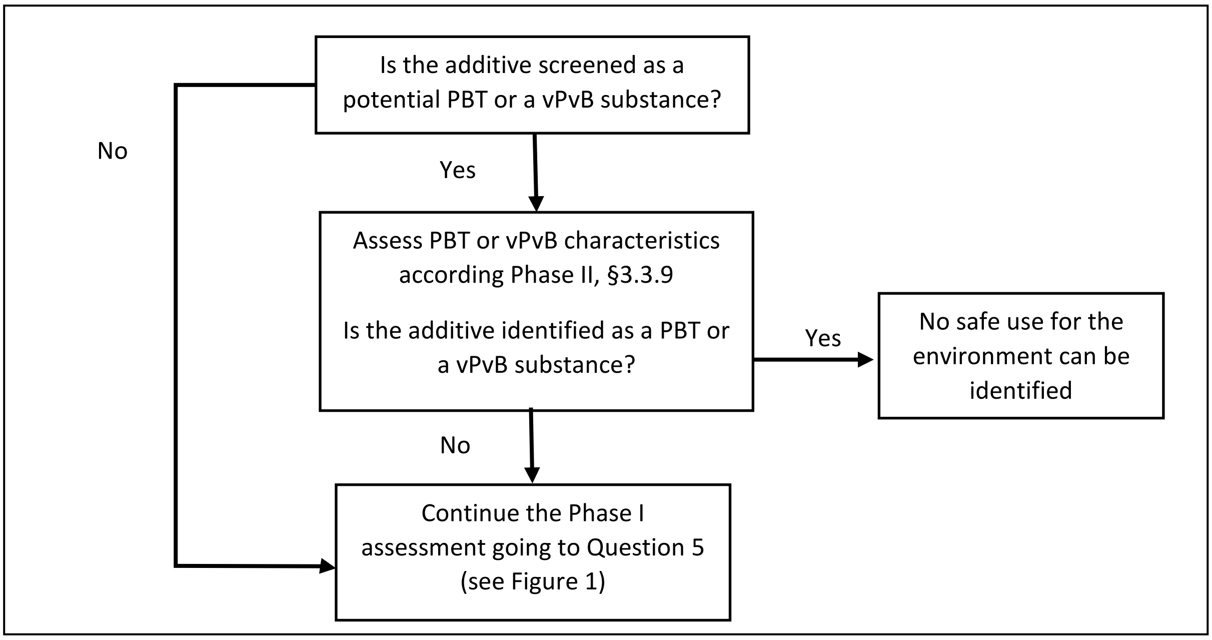


Figure 2: Decision scheme for assessing PBT or vPvB properties of the feed additive.

2.5. Question 5: has the feed additive a specific mode of action of concern or may it potentially accumulate in soil due to multi-year application?

Coccidiostats and histomonostats are chemicals with a specific toxic mode-of-action against harmful protozoa. Currently they are authorised as feed additives in poultry and rabbit feed and, consequently, may be toxic to non-target organisms in environments that receive poultry/rabbit manure. A Phase II ERA is expected for these feed additives (see Section 3). Other substances, on the basis of toxicological studies on laboratory animals or other evidence, may show toxicological properties *in vivo* that are of potential concern for environmental biota at sub-lethal concentrations, e.g., reproductive toxicity. Substances that hardly dissipate in the environment of concern may accumulate in the receiving compartment(s), which can only be properly assessed when information on long-term fate is available. Therefore, when there is already evidence (either experimental or by screening) that a feed additive is not degradable and hardly dissipates, e.g. metals or other chemical elements that are excreted at amounts that can significantly increase the concentration in environmental compartments (see Question 2), these substances have to be assessed in Phase II.

2.6. Question 6a: Is the predicted environmental concentration of the feed additive used in terrestrial livestock species below a trigger value?

When excreta from livestock are applied on land, the use of feed additives can lead to contamination of soil, ground water and surface water (via drainage and run-off).

The PECs used in Phase I would arise considering all excreted compounds being spread on land and other specified assumptions (see Sections 2.6.1 and 2.6.2) which reflect in summary worst-case conditions.

If PEC for soil (PEC_{soil}) (default: 5 cm depth) is less than 10 µg/kg dry weight; and

PEC for pore water (PEC_{pw}, surrogate for PEC groundwater) (default: 20 cm soil depth) is less than 0.1 µg/l,

the substance is considered not to pose a risk for the environment, and therefore no further assessment is necessary, unless there is available scientific evidence that it could represent a risk for human health and/or the environment.

2.6.1. Calculation of PEC in soil (PEC_{soil})

The amount of manure/slurry containing the feed additives allowed to be spread on land depends on the nitrogen content of the manure and the annual nitrogen load. Based on the data on feed intake and nitrogen content in manure, the maximum amount of parent compound per kg nitrogen excreted can be calculated by multiplying the concentration of the additive in feed with the feed consumption and dividing it by the corresponding nitrogen excretion. In Table 1, the feed intake and corresponding nitrogen excretion is given for the more relevant food-producing species/categories. Other data can be used if justified.

For a worst-case estimation of the concentration in soil, the following assumptions are made:

- The additive is continuously applied at the maximal recommended dose (as proposed by the applicant) to the feed of the target animal;
- Total intake of the active substance is considered to be excreted as parent compound;
- The current annual nitrogen load standard for slurry/manure spread on farm/livestock unit in nitrogen vulnerable areas is 170 kg N/ha per year (EU nitrate directive 91/676/EEC). The annual nitrogen emission standard is an average value that might be applied on a farm per year. According to the code of good agricultural practices, the emission to particular non vulnerable fields with crops/grass could exceed this value. It is recognised that in current agricultural practice in EU this average value could be exceeded and a different value could be considered (See Appendix G);
- There is no dissipation of the parent compound during storage and spreading of slurry/manure;
- The standard assumption, when slurry/manure is spread on land, is that the additive is mixed in the soil up to 5 cm depth.

Table 1: Default values for feed intake and nitrogen excretion (see in Appendix H the assumptions made in the different calculations)

Animals	Body weight start-end (kg)	Productive cycles/year⁽¹⁾	Feed intake (kg/animal place per year)⁽²⁾	Nitrogen excreted (kg/animal place per year)
Piglet	7-30	7.4	296	4
Pig for fattening	30-115	3.2	800	9
Sow with piglets	200	2.4	1140	23
Cattle for fattening	250-630	1.2	4050	54
Veal calf	45-250	1.5	730	11
Dairy cow (3)	650	0.92	6584	125
Lamb for fattening	4-32	1.5 (7)	273	5
Sheep for fattening	15-55	1.5 (7)	267	5
Meat sheep	60	1	607	10
Dairy sheep	60	1	580	10
Dairy goat	50	1	714	16.4
Chicken for fattening	0.045-2.2	6.5	22	0.33
Laying hen(4)	1.4-2	0.84	42	0.8
Turkey for fattening(5)	0.05-10(f)/16(m)	2.6	70	1
Rabbit for fattening	0.9-3.1	4.8	30	0.5
Horse(6)	500	1	3650	58
Horse for fattening	270-480	1.5(7)	2385	43

(1): Number of productive cycles per animal place during a year.
(2): Feed containing 88% DM in non-ruminant species and 100% DM in ruminant species.
(3): Considering a milk production of 8,000 kg/year.
(4): Considering a production of 300 eggs/year.
(5): Considering an average final weight (males (m) and females (f)) of 13 kg at slaughter.
(6): Considering a mature horse in maintenance phase.
(7): Calculated considering the seasonality of the oestrus of this species.

Feed intake and the nitrogen excretion are dependent on the size, production level and age of the animal. Typically both the intake and the excretion are calculated over a position in a stable ('animal place') during one year.

If the feed additive is intended for use in a livestock species or animal category that is not listed in Table 1, the proposed value should be motivated by providing scientific evidence to allow EFSA evaluating the proposal.

The following equations should be used to calculate PEC in manure and soil:

$$PEC_{manure} = \frac{C_{add} \cdot FI_{total}}{N_{excreted}}$$

$$PEC_{soil_{dw}} = \frac{PEC_{manure} \cdot Q}{RHO_{soil} \cdot CONV_{area\ field} \cdot DEPTH_{field}}$$

where:

Symbol	Parameter	Default Value*	Unit
Input			
C_{add}	Concentration of the additive in feed		mg/kg complete feed
FI_{total}	Total feed intake (DM) per animal per year		kg feed/year
$N_{excreted}$	Total N excretion per animal per year		kg N/year
RHO_{soil}	Bulk density of (dry) soil	1,500	kg/m^3
$DEPTH_{field}$	Mixing depth with soil	0.05	m
$CONV_{area\ field}$	Conversion factor for the area of the agricultural field	10,000	m^2/ha
Q	Annual nitrogen emission standard	170	kg N/ha
Intermediate results			
PEC_{manure}	Concentration of the additive (parent compound) in manure expressed per amount nitrogen		mg/kg N
Output			
PEC_{soildw}	Concentration of the additive (parent compound) in soil (dry weight)		mg/kg soil$_{dw}$

* The use of the indicated default values in the equations is recommended. Reasons for any deviations from these values should be given by the applicant.

Using these formulas, the concentration of a feed additive (mg/kg feed) that would correspond to a PEC_{soil} below the trigger value for the different species can be calculated back as shown in Appendix F.

2.6.2. Estimation of $PEC_{groundwater}$

Several numerical models are available to calculate groundwater concentrations of agrochemicals (mainly for pesticides). These models, however, require a characterisation of the soil to a high level of detail. This makes these models less appropriate for a preliminary assessment. Therefore, as an indication for potential groundwater levels, the concentration in pore water of agricultural soil is taken. PEC in groundwater is set equal to PEC in pore water. It should be noted that this is a worst-case assumption, neglecting transformation and dilution in deeper soil layers.

The PEC of pore water (PEC_{pw}) is calculated using the approach described in REACH guidance R16, (ECHA, 2016).

In this screening model, partitioning depends on equilibrium sorption to solids, no saturation at binding places and steady-state conditions. This model provides a worst-case estimate of the pore water concentrations as movement, dilution, desorption, transformation, weather or crops are not considered. Soil is defined through compartment volumes for solids, water and air, dry bulk density and texture (mineral and organic fraction). The soil depth for calculation of the PEC_{soil} used for calculating the PEC_{pw} is set at 20 cm.

The model calculation of the concentration in pore water is as follows:

$$PEC_{manure} = \frac{C_{add} \cdot FI_{total}}{N_{excreted}}$$

$$PEC_{soil\,ww} = \frac{PEC_{manure} \cdot Q}{RHO_{soil} \cdot CONV_{area\ field} \cdot DEPTH_{field}}$$

$$K_{air-water} = \frac{VP \cdot MOLW}{SOL \cdot R \cdot TEMP}$$

$$Kp_{soil} = Foc_{soil} \cdot Koc$$

$$K_{soil-water} = Fair_{soil} \cdot K_{air-water} + F_{water-soil} + F_{solidsoil} \cdot \frac{Kp_{soil}}{1000} \cdot RHO_{solid}$$

$$PECporewater = \frac{PECsoil\ ww * RHOsoil}{Ksoil\ water * 1000}$$

where:

Symbol	Parameter	Default Value*	Unit
Additive properties			
C_{add}	Concentration of the additive in feed		mg/kg complete feed
VP	Vapour pressure		Pa
MOLW	Molar mass		g/mol
SOL	Water solubility		mg/l
K_{oc}‡	Organic carbon normalised partition coefficient		dm^3/kg
Substance independent input			
RHO_{soil}	Bulk density of (wet) soil	1,700	kg/m^3
$DEPTH_{field}$	Mixing depth with soil	0.2	m
RHO_{solid}	Bulk density of soil solids	2,500	kg/m^3
$Fair_{soil}$	Fraction air in fresh field soil	0.2	m^3/m^3
$F_{water\text{-}soil}$	Fraction water in fresh field soil	0.2	m^3/m^3
$F_{solidsoil}$	Fraction solids in fresh field soil	0.6	m^3/m^3
Foc_{soil}	Weight fraction organic carbon in dry weight soil	0.02	kg/kg^1
TEMP	Temperature at air-water interface	285	°K
R	Gas constant	8.314	Pa m^3/mol/°K
FI_{total}	Total feed intake (DM) per animal in a year	See Table 1	kg feed/year
$N_{excreted}$	Total N excretion per animal in a year	See Table 1	kg N/year
Q	Annual nitrogen emission to soil	170	kg N/ha
$CONV_{area\ field}$	Conversion factor for the area of the agricultural field	10,000	m^2/ha
Intermediate results			
$K_{soil\text{-}water}$	Partition coefficient solids and water in soil (v/v)		m^3/m^3
Kp_{soil}	Partition coefficient solids and water in soil (v/w)		dm^3/kg

$K_{air-water}$	Partition coefficient air and water in soil	m^3/m^3
Output		
PEC_{manure}	Concentration of the additive (parent compound) in manure expressed per amount nitrogen	mg/kg N
PEC_{soilww}	Concentration of the additive (parent compound) in soil (wet weight)	mg/kg $soil_{ww}$
PEC_{pw}	Concentration of the additive (parent compound) in pore water	mg/l

* The use of the indicated default values in the equations is recommended. Reasons for any deviations from these values should be given by the applicant.

‡ Where no measured K_{oc} value is available, in the Phase I assessment estimation techniques can be used based on correlation with the K_{ow} or water solubility given in OECD guideline 106 (Soil Adsorption/Desorption) or from a quantitative structure-activity relationships (QSAR) calculation as described in Appendix D. When experimental data is available, explanations on how to select the K_{oc} are given in Section 3.3.1.

2.7. Question 6b: Is the predicted environmental concentration of the feed additive used in aquaculture below a trigger value?

Feed additives used in aquaculture can result in contamination of sediment and water.

The method to calculate the PEC in sediment and water varies for the different European fish production systems: sea cages versus land-based aquaculture (ponds, tanks and recirculation systems). In aquaculture operations involving the use of sea cages, benthic organisms (living in or on sediments) are considered to be most at risk whereas both waterborne exposure of pelagic organisms (living in the water column) and benthic organisms present the main risk from land-based fish farms that discharge to shallow freshwater ecosystems.

The PECs used in Phase I should be calculated considering all excreted compounds being dispersed to sediment and water and other specified assumptions (see Sections 2.7.1 and 2.7.2) which reflect in summary worst case conditions.

The organic carbon content of the sediment may influence the bioavailability and therefore the toxicity of the test substance. Therefore, for comparison of sediment tests, the organic carbon content of the test sediment should be within a certain range. The OECD guideline 218 for the test with *Chironomus* using spiked sediment recommends an organic carbon content of the test sediment of 2% (±0.5%) (EMA, 2016).

If PEC for sediment:

- (PEC_{sed}) (default: 5 cm depth assuming 2±0.5% organic carbon (OC)) is less than 10 µg/kg dry weight; and
- PEC for surface water (PEC_{sw}) is less than 0.1 µg/l

the substance is considered not to pose a risk for the environment, and therefore no further assessment is necessary.

2.7.1. Calculation of PEC in the sediment (PEC_{sed}) for sea cages

The calculation of PEC_{sed} is considered a realistic worst-case value that covers the use of feed additives for a wide range of fish species. It should be calculated as follows:

$$PC_{faeces} = C_{add} \times CF$$

$$PEC_{sed} = \frac{PC_{faeces} \times \cdot k_{dep} \times T_{production}}{RHO_{solid} \times F_{solid} \times DEPTH_{sed}}$$

where:

Symbol	Parameter	Default value*	Unit
Input			
C_{add}	Concentration additive in feed		mg/kg complete feed
CF	Conversion factor (kg feed to kg total carbon in faeces)	15.1‡	kg/kg carbon
k_{dep}	Maximum deposition rate of faeces	0.01¥	kg carbon/m^2 per day
$T_{production}$	Number of production days	365	day
RHO_{solid}	Bulk density of solids	2,500[1]	kg/m^3
$DEPTH_{sed}$	Mixing depth in sediment	0.05	m
F_{solid}	Volume fraction of solids in fresh field collected sediment	0.2	m^3/m^3
Output			
PC_{faeces}	Concentration of the additive (parent compound) in the carbon fraction of faeces		mg/kg carbon
PEC_{sed}	Highest initial concentration of additive in dry weight sediment		mg/kg

* The use of the indicated default values in the equations is recommended. Reasons for any deviations from these values should be given by the applicant.
‡ Concentration of the additive in feed (C_{add}) given in mg/kg feed has to be converted in mg/kg C feed (2.06). Subsequently, mg/kg[1] C feed is converted to into mg/kg C faeces (7.3), hence the total conversion is 2.06 × 7.3=15.1
¥ According to Hansen et al., 1991 ; Karakassis et al., 2002 ; Corner et al., 2006 ; Holmer et al., 2006 ; Kutti et al., 2007
1 Assumed to be similar for soil and sediment (see section 2.6.2.)

2.7.2. Calculation of PEC in surface water from aquaculture (PEC_{swaq}) in raceway/pond/tanks and recirculation systems

The PEC_{swaq} can be calculated as follows:

$$PEC_{swaq} = \frac{C_{add} \cdot FR}{Flow \cdot DF}$$

where:

Symbol	Parameter	Unit
Input		
C_{add}	Concentration of the additive in feed	mg/kg complete feed
FR	Feed Ration	kg feed/kg fish per day
Flow	Water flow rate through the system	l/kg fish per day
DF	Dilution Factor	10
Output		
PEC_{swaq}	Highest initial concentration of additive (parent compound) in surface water	mg/l

In Phase I it is assumed that the total amount of the additive in feed is released into the aquaculture system (i.e. there is no retention in "sludge" such as water material that is filtered or settles out within the facility).

For feed daily ration and water flow rate, the following default settings are proposed for some fish species commonly farmed in Europe. The information of Table 2 for sea bass, sea bream and turbot refers to their breeding in inland aquaculture systems. For species not listed in table 2, the applicant may propose other values and provide a justification.

Table 2: Feed ration and water flow rate in fish farming in Europe

Fish types	Feed Ration (kg feed/kg fish per day)	Water flow rate (L/kg fish and day)
Salmon	0.01[1]	865[4]
Rainbow trout	0.02	1400[2]
Sea bass/Sea bream	0.01[3]	400[3]
Turbot	0.01[3]	720[3]

[1] Bailey, 2003
[2] http://www.fao.org/fishery/culturedspecies/Oncorhynchus_mykiss/en#tcNC008F (2005)
[3] Hussenot et al., 1998
[4] Mattilsynet (Norwegian Food Safety Authority), 2004

3. Phase II assessment

The aim of Phase II is to assess the potential for additives to affect non-target species in the environment, including both aquatic and terrestrial species or to reach deeper groundwater at levels above a concentration of 0.1 µg/l. It is not practical to evaluate the effects of additives on every species in the environment that may be exposed to the additive following its administration to the target species. Therefore, certain taxa/endpoints are recommended to be tested and intended to serve as surrogates or indicators for the range of species/functions present in the environment.

The Phase II assessment is based on a risk quotient approach, where the calculated PEC and Predicted No Effect Concentration (PNEC) values for each compartment of concern should be compared. The PNEC is determined from experimentally determined endpoints divided by an appropriate assessment (safety) factor. The value of the assessment factor (AF) is dependent on the amount of accurate and relevant data available, associated uncertainties and harmonisation requirements between different legislations.

For the effect assessment (e.g. PNEC derivation), the tier 1 usually is based on the basic dossier requirements. Since lower tiers should be more conservative than higher tiers, effect estimates (e.g. PNECs) generated at higher tiers should be higher than those at lower tiers. Consequently, higher tier information can be used to validate/calibrate lower tiers. Ideally, the consistency of the different tiers within an ERA scheme should be evaluated for a number of benchmark feed additives.

If the feed additive is a metal salt and data for the same metal but a different salt is available, these can be used in the PNEC derivation when scientifically justified and properly documented.

The Phase II assessment is based on a tiered approach (Figure 3). The first tier, Phase IIA, makes use of a limited number of fate and effect studies to produce a conservative assessment of risk based on exposure and effects in the environmental compartment of concern. This would also mean that the PECs from Phase I have to be recalculated (PEC_A) using the information on metabolism in the target animal(s) and experimental fate data, i.e. adsorption and degradation.

In all tiers (Phases IIA to IIC) a comparison should be made between the PEC and the PNEC (or threshold value for the groundwater):

- Phase IIA: If the PEC_A is lower than the $PNEC_I$ values and the trigger value for groundwater is not exceeded, no further assessment is required, unless accumulation is expected (for further details see Section 3.3);
- Phase IIB: If the $PEC_A/PNEC_I$ is ≥ 1, a more refined PEC (= PEC_B) can be calculated based on additional data not yet considered (for further details see Section 3.4);
- Phase IIC: If the $PEC_A/PNEC_I$ or $PEC_B/PNEC_I$ ratio predicts a potential risk (ratio > 1), a more refined PNEC (=$PNEC_R$) can be derived to better estimate the environmental risks (for further details see Section 3.5).

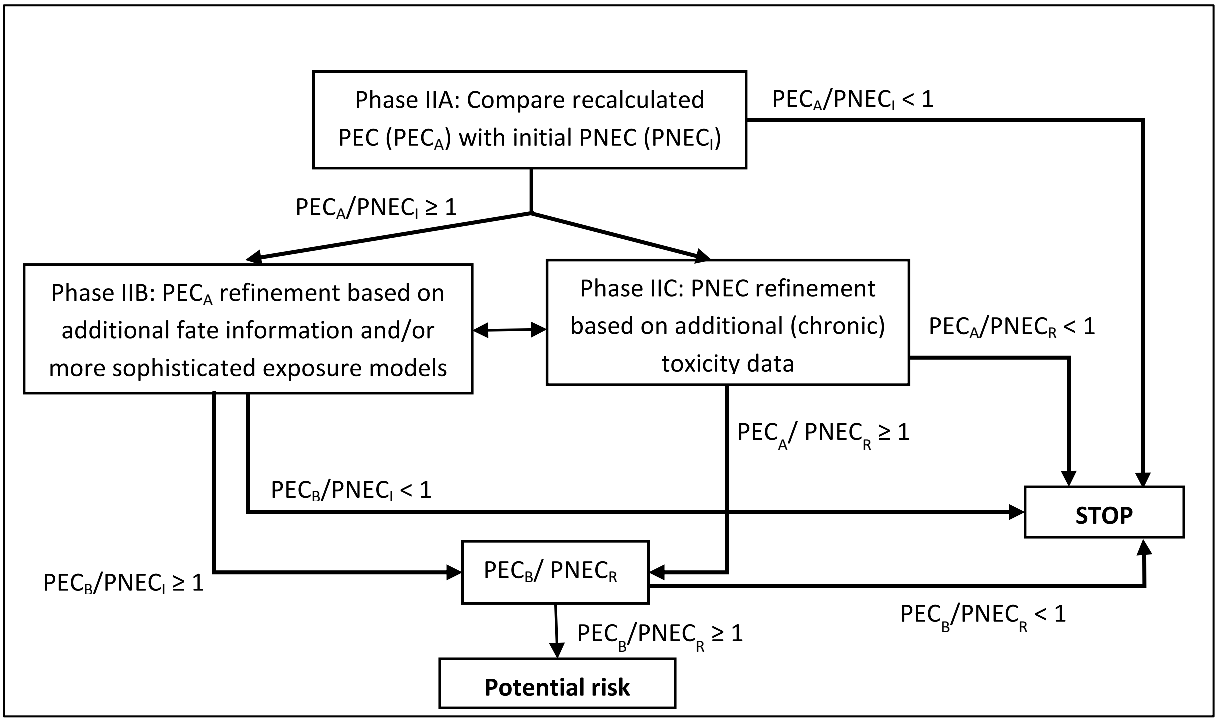


Figure 3: Phase II decision tree for the environmental risk assessment of soil and aquatic compartment for terrestrial animals (PEC_A and PEC_B concern PECs for soil, groundwater, surface water and sediment recalculated using procedures described in sections 3.3.1 – 3.3.5 and section 3.4., respectively; $PNEC_I$ and $PNEC_R$ are initial and refined PNECs calculated using procedures described in sections 3.3.6 and 3.5, respectively)

The comparison of the PEC to PNEC estimates is based on the following principles (see Sections A.5 and 7 of Appendix A):

1. The effect assessment and exposure assessment is based on the same ecotoxicologically relevant type of concentration;
2. When the PNEC is derived from acute toxicity data, only the predicted environmental peak concentration (PEC_{max}) is used for comparison.
3. When the PNEC is derived from chronic toxicity data, the PEC_{max} can be considered as a precautionary worst-case approach. Alternatively, the time-weighted average (PEC_{twa}) may be used if:
    a. Reciprocity of effects is demonstrated/likely;
    b. The chronic toxicity estimates (EC_{10} or NOECs) on which the PNEC is based are expressed in terms of (geometric) mean concentrations during the exposure period of the test; in case measured concentrations in the course of the experiment are within 20% of nominal, the nominal concentration can be used as a proxy of the mean concentration.
    c. The time-frame of the PEC_{twa} estimate should be ≤ than the duration of the exposure periods in the chronic toxicity tests that drive the PNEC.
4. Toxicity data that are expressed in terms of initial exposure concentration and show a decline larger than 20% in the course of the experiment, may be used to derive a PNEC if in the ERA this PNEC is compared with the PEC_{max} and it is likely/plausible that the decline in exposure is not faster in the toxicity tests than that predicted for the environment. To demonstrate this, either validated exposure models or chemical monitoring data are required that enable to characterise the dynamics in exposure concentration of the feed additive for the environmental compartment of concern. If these models/data are not available a precautionary approach is advocated by expressing the laboratory toxicity estimates in terms of mean (e.g. geometric mean or time-weighted average) exposure concentration during the test and by selecting the PEC_{max}.

In case of difficult substances, consider OECD Series on Testing and Assessment (Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures). If the problem cannot be solved using this guidance, an additional environmentally more realistic study may be requested.

3.1. Physico-Chemical properties studies

In order to evaluate the fate and toxicity of the feed additive, some basic physico-chemical properties are needed. The studies required are reported in Table 3 (EMA, 2005).

Table 3: Physico-chemical properties studies in Phase IIA (EMA, 2005)

Study	Guideline
Water solubility	OECD 105
Dissociation constants in water	OECD 112
UV-Visible absorption spectrum	OECD 101
Vapour pressure [a]	OECD 104
n-Octanol/water partition coefficient	OECD 107, 117 or OECD 123
Melting point/melting range [b]	OECD 102

a. Calculation only, though a study is recommended when other physical-chemical properties, e.g. molecular weight, melting temperature, thermogravimetric analysis suggest that the vapour pressure may exceed 10^{-5} Pa at 20°C.
b. This parameter is not strictly needed in the assessment. Nevertheless, melting point/melting range together with vapour pressure provide information on the distribution of the substance within and between the environmental media (water, soil and air).

Water solubility provides information on how likely the feed additive will be distributed by the hydrological cycle and gain access to living organisms. It is also important to set up test conditions for a range of fate (e.g. biodegradation, bioaccumulation) and effects studies.

Dissociation constants in water may affect the adsorption of the substance on soils and sediments and absorption into biological cells. It may also be an important factor in deciding which method or conditions should be used to determine the octanol-water partition coefficient and soil adsorption partition coefficient (see section 3.2).

UV-Visible absorption spectrum gives information on the potential of a substance to photodegrade and/or to be phototoxic under environmental relevant conditions.

The n-Octanol/water partition coefficient (K_{ow}) is used to estimate the environmental partitioning, e.g. adsorption and bioaccumulation. Some precautions must be taken regarding the use of the shake-flask method (OECD 107) or the high performance liquid chromatography (HPLC) method (OECD 117) to determine log K_{ow} for very lipophilic compounds. These are outlined in the Globally Harmonized System of Classification and Labelling of Chemicals:

"*The shake-flask method is recommended when the log K_{ow} value falls within the range from –2 to 4. The shake-flask method applies only to essential pure substances soluble in water and n-octanol. For highly lipophilic substances, which slowly dissolve in water, data obtained by employing a slow-stirring method are generally more reliable. Furthermore, the experimental difficulties, associated with the formation of microdroplets during the shake-flask experiment, can to some degree be overcome by a slow-stirring method where water, octanol, and test compound are equilibrated in a gently stirred reactor. With the slow-stirring method (*OECD 123*) a precise and accurate determination of K_{ow} of compounds with log K_{ow} of up to 8.2 is allowed. As for the shake-flask method, the slow-stirring method applies only to essentially pure substances soluble in water and n-octanol. The HPLC method, which is performed on analytical columns, is recommended when the log K_{ow} value falls within the range 0 to 6. The HPLC method is less sensitive to the presence of impurities in the test compound compared to the shake-flask method.*"

It should also be emphasised that the log K_{ow} for ionisable substances should be measured on the non-ionised form at environmentally relevant pHs.

3.2. Environmental fate studies

Biodegradation studies should be performed in soil for feed additives intended for use in terrestrial species and in aquatic systems for feed additives intended for aquatic animals. The Soil Adsorption/Desorption test should be used for additives for both terrestrial and aquatic species as long as there is no validated test for sediment. Table 4 describes the studies required for Phase IIA (EMA, 2005).

Table 4: Environmental fate studies for phase IIA (EMA, 2005)

Study	Guide line
Soil Adsorption/Desorption	OECD 106/121
Soil Biodegradation (route and rate)*	OECD 307
Photolysis (optional)	OECD 316
Hydrolysis (optional)	OECD 111

*Recommended only for the terrestrial branch.

3.2.1. Soil adsorption/desorption

Adsorption/desorption studies should report both the sorption/desorption coefficient normalised to carbon content (K_{oc}) and the dissociation constant (K_d) values for a range of soils. The OECD 121 guideline to determine the log K_{oc} by means of HPLC should be used with care. For polar compounds especially, this method is not fully validated and may provide unreliable K_{oc} values. Also log K_{oc} values higher than 5.6 should not be considered to be reliable. For this reason, the OECD 106 test method is recommended. As a minimum five different soils or sediments should be selected to investigate the dependency of the Koc value to the different soil properties. Depending on the dissociation constant these substances could dissociate into ionic species around environmental pH values, which may have significantly different water solubilities and partition coefficients than the non-dissociated species. If the acid dissociation constant (pK_a) value is within the environmentally-relevant pH range, the selected soils should cover a wide range of pH, in order to evaluate the adsorption of the substance in its ionised and unionised forms as recommended in the OECD TG 106.

Other soil components with polar and/or charged surfaces may also act as sorbents, e.g. cations can often sorb to clay particles instead of organic material.

In most cases the K_{oc} can be used to estimate the sorption of the feed additive to soil or sediment, but a direct estimation of the $K_{soil\text{-}water}$ can also be useful. Especially for ionophores, it is important to know the main factors that govern the sorption of the molecule to soil or sediment. For compounds that are mainly sorbed to clay, the partition coefficient (K_p) can be calculated for a standard soil or sediment containing 20% clay. When appropriate, models need to be adapted to account for additional sorbents and pH-dependence of sorption.

3.2.2. Soil biodegradation and degradation in aquatic compartment

The soil degradation simulation study (OECD TG 307) is recommended for feed additives used in livestock. When feed additives are used in aquaculture a water/sediment degradation simulation study (OECD TG 308) is more relevant. For feed additives used in mariculture it may be more appropriate to do this study under saltwater conditions.

3.2.3. Photo-degradation and hydrolysis

Investigation of photolysis is optional as it is expected that there will be little direct exposure of the feed additive to light in the manure or soil matrix and that therefore photodecomposition does not play a significant role in the overall degradation of feed additives here.

Information on hydrolysis might only be relevant when this process will dominate the degradation of the feed additive in the aquatic environment.

3.3. Phase II A

In Phase IIA the PEC_A recalculated as described below is compared with a $PNEC_I$ based on minimum data requirements for feed additives. The $PNEC_I$ derivation is largely based on short-term toxicity tests.

3.3.1. Phase II A PEC_{soil} calculation

In Phase IIA the PEC_A is calculated based on the methodology described in Section 2 taking the following into account:

- The measured concentration of active substance/metabolites of concern in manure following administration of the additive to livestock animals at the proposed dose level. This calculation should include consideration of dosage rates and amount of excreta produced. Metabolites representing less than 10% of the administered dose can be subtracted from the total dose administered. In addition, the biological activity of metabolites compared to the parent compound should be considered. This procedure will result in the calculation of the fraction of the administered dose still considered to be active.
- The adsorption/desorption of the active substance/metabolites of concern onto soil is preferentially determined by studies in soil.
- Degradation in soil: In accordance to the EFSA guidance (EFSA, 2014) it is recommended to use the geometric mean of the degradation rates as inputs in the exposure models. In case there are indications the degradation rate depends on soil properties such as clay or pH, the FOCUS guidance (FOCUS, 2009) should be followed to determine the appropriate PECs. If a high persistence in soil is anticipated (time to degradation of 50% of original concentration of the compound (DT_{50}) > 60 days at 12°C), the potential for accumulation should be considered. If data at 12°C are not available, data obtained at 20°C could be extrapolated using the Arrhenius equation (activation energy: 65.4 kJ/mol according to the EFSA guidance for use in FOCUS (EFSA, 2008b)). Consequently, a factor of 2.12 was used to calculate the DT_{50} at 12°C (DT_{50} at 12°C = DT_{50} at 20°C * 2.12).
- Ploughing depth: In some countries manures are mainly spread on and mixed into arable land used for crop production, e.g. Belgium, Denmark, Finland, France, Germany, Italy and Spain. In other countries, e.g. Greece, Ireland and UK, it is common practice to distribute manure directly onto grassland (Burton and Turner, 2003). These differences prevent a general refinement of the 5 cm mixing depth used in Phase I (EMA, 2008). Therefore, concentrations in soil should be calculated for application in grassland ($PEC_{soil,grassland}$; depth of 5 cm) but possible dilution of the feed additive due to ploughing ($PEC_{soil,\ arable\ land}$; 20 cm soil depth) will be taken also into account.

3.3.1.1. Recalculation based on metabolism

When metabolism data are considered, the $PEC_{soil\ A}$ is calculated based on the methodology described in Phase I and recalculated as shown:

$$PEC_{soil\ A} = PEC_{soil\ initial} \cdot Fa$$

where:

Symbol	Parameter	Unit
$PEC_{soil\ A}$	Refined concentration of the additive (parent compound) in dry soil	mg/kg
$PEC_{soil\ initial}$	Concentration of the additive (parent compound) in dry soil in Phase I	mg/kg
Fa*	Fraction of the dose considered to be active	-

* [value between 0 and 1]

When the application covers several target species/categories, it is recognised that it may be unrealistic to expect studies in all potential target species for which application is made, especially when the application is for all animal species. Therefore, inter-species extrapolation of data can be applied. The applicant is referred to the EFSA FEEDAP Panel (2017) guidance on the assessment of the safety of feed additives for the consumer (Section 2.1.1.1) to select the most representative species to be investigated.

3.3.1.2. Recalculation based on degradation in soil

If the feed additive is not expected to degrade within a year (i.e. DT_{50} > 60 days at 12°C), the potential for residues to accumulate in soil should be considered. In those cases, the $PEC_{soil\ plateau}$ at steady state should be calculated at the start of Phase IIA as follows:

$$PEC_{soil\,1year} = PEC_{soil\,initial} \times e^{\left(\frac{(-0.693 \times 365)}{DT_{50}}\right)}$$

$$Fd = \frac{\left(PEC_{soil\,initial} - PEC_{soil1\,year}\right)}{PEC_{soil\,initial}}$$

$$PEC_{soil\,A\,plateau} = \frac{PEC_{soil\,initial}}{Fd}$$

where:

Symbol	Parameter	Unit
Input		
DT_{50}	Half-life of additive (parent compound) in soil at 12 °C	day
$PEC_{soil\ initial}$	Concentration of the additive (parent compound) immediately after spreading in dry soil	mg/kg
Intermediate results		
Fd	Fraction of additive (parent compound) degraded in 1 year	-
Output		
$PEC_{soil\ 1\ year}$	Concentration of the additive (parent compound) 1 year after spreading in dry soil	mg/kg
$PEC_{soil\ A\ plateau}$	$PEC_{soil\ A}$ at plateau concentration in dry soil	mg/kg

The PEC in soil can be refined based on either information related to the metabolism of the substance in the target animals or degradation in manure or soil. In every case, kinetic results such as the degradation rates and degradation half-lives should correspond to an environmentally relevant temperature, i.e. by default 12 ºC (ECHA, 2017c: Guidance on Information Requirements and Chemical Safety Assessment Chapter R.7b: Endpoint specific guidance, Section 7.9.4.1).

3.3.1.3. Recalculation based on degradation in soil under multiple applications

Refinement of PEC_{soil} based on soil degradation data is possible when it is realistic to assume that manure is spread in more than one spreading event. In that case, the concentration calculated after the last spreading event should be taken.

In the case of arable land, manure/slurry is usually applied to fulfil the permissible limit during a single, annual application event. This partly reflects the fact that the presence of a crop will prevent applications of manure/slurry throughout much of the year.

In the case of grassland, it is more typical to make a number of applications of manure/slurry throughout the year. It is up to the applicant to provide information to support the number of spreading events which have been taken to occur on grassland.

As the storage capacity shows a large variation among the different EU Member States, it is recommended to set the storage capacity/time equal to the production period of the target animal up to three months, unless the number of cycles is more than four per year. In this case the storage time is set equal to the period of the cycle. Similar default values on storage time (days) are indicated in the *Guideline on environmental impact assessment for veterinary medicinal products in support of the VICH guidelines GL6 and GL38, Rev. 1* (EMA, 2016).

The following formula can be used to calculate the PEC_{soil} after the last spreading event:

$$PEC_{soilA} = PEC_{soil\,single\,event} \cdot \frac{1 - F_{rs}^{(Nspreading)}}{1 - F_{rs}}$$

$$F_{rs} = e^{-k \cdot Tinterval\ spreading}$$

$$k = \frac{\ln 2}{DT_{50}}$$

where:

Symbol	Parameter	Unit
Input		
$PEC_{soil\ single\text{-}event}$	Concentration of the additive (parent compound) in dry weight soil immediately after spreading	mg/kg
$N_{spreading}$	Number of spreading events	
$T_{interval\ spreading}$	Time between spreading events	day
DT_{50}	Half-life of additive (parent compound) in soil	day
K	Rate constant	
Intermediate results		
F_{rs}	Fraction remaining in soil after time $T_{interval\ spreading}$	
Output		
$PEC_{soil\ A}$	Refined Concentration of the additive (parent compound) in dry weight soil after last spreading event	mg/kg

3.3.2. Phase II A PEC groundwater calculation

Based on the experimentally determined K_{oc} value, the concentration in groundwater is recalculated using the same methodology as used in Phase I (see section 2.6.2).

In accordance to the EFSA guidance (PPR Panel, 2014a) it is recommended to use the geometric mean of the Koc values as inputs in the exposure models. In case there are indications the adsorption depends on soil properties such as clay or pH, the FOCUS guidance (FOCUS, 2009) should be followed to determine the appropriate PECs;

If the feed additive is not expected to degrade within a year (i.e. DT50 > 60 days at 12°C), the potential for residues to accumulate in soil should be considered by using a PEC soil plateau. This can be calculated by dividing the PEC soilww by the fraction of additive (parent compound) degraded in 1 year (Fd) as calculated in section 3.3.1.2.

$$PEC\ pore\ water\ plateau = \frac{\frac{\text{PEC soil ww}}{\text{Fd}} * \text{RHO soil}}{\text{Ksoil water} * 1000}$$

3.3.3. Phase II A PEC surface water calculation

As a first estimate of the concentration in surface water resulting from run-off or drainage, it is assumed that one part run-off/drainage water will be diluted by two parts receiving water (Montforts, 1997, 1999). The concentration in run-off/drainage water is assumed to be equal to the concentration in pore water as calculated in the previous Section 3.3.2.

$$PEC_{swA} = \frac{PEC_{pw\,A}}{3}$$

where:

Symbol	Parameter	Unit
Input		
$PEC_{pw\,A}$	Concentration of the additive (parent compound) in pore water	mg/l
Output		
$PEC_{sw\,A}$	Concentration of the additive (parent compound) in surface water	mg/l

If the feed additive is not expected to degrade within a year (i.e. DT50 > 60 days at 12°C), the potential for residues to accumulate in soil should be considered. In that case the PEC pore water plateau should be used as calculated in section 3.3.2.

$$PEC_{sw\ plateau\ A} = \frac{PEC_{pw\ plateau\ A}}{3}$$

3.3.4. Phase II A PEC sediment calculation

In Phase IIA the $PEC_{sed\ A}$ is calculated from $PEC_{surfacewater\ A}$ using the Equilibrium partitioning EqP concept (Ref) as follows:

$$PEC_{sed\ A} = \frac{K_{susp-water}}{RHO_{susp}} \times PEC_{surfacewaterA} \times 1000 \times CONV_{susp}$$

$$K_{susp-water} = Fwater_{susp} + \left(Fsolid_{susp} \times \frac{Kp_{susp}}{1000} \times RHO_{solid} \right)$$

$$CONVsusp = \frac{RHO\ susp}{Fsolid\ susp * RHOsolid}$$

$$Kp_{susp} = Foc_{susp} \times K_{oc}$$

where:

Symbol	Parameter	Default Value*	Unit
Input			
$K_{susp\text{-}water}$	suspended matter**-water partition coefficient		m^3/m^3
RHO_{susp}	Bulk density of (wet) suspended matter***	1150	kg/m^3
RHO_{solid}	Bulk density of solids	2500	kg/m^3
$PEC_{surfacewater\ A}$	Predicted environmental concentration for surface water		mg/L
$CONV_{susp}$	Conversion factor for suspended matter concentrations: wet weight to dry weight		kg_{ww}/kg_{dw}
$Fwater_{susp}$	Volume fraction of water in suspended matter	0.9	m^3/m^3
1000	Conversion for litre to m^3		L/m^3
$Fsolid_{susp}$	Volume Fraction of solids in suspended matter	0.1	m^3/m^3 of water-solid slurry
Kp_{susp}	Partition coefficient solids and water in suspended matter (v/w)		L/kg

K_{oc}	Organic carbon partition coefficient		L/kg[1]
Foc_{susp}	Weight fraction organic carbon in suspended solid	0.1	kg/kg
Output			
$PEC_{sed\ A}$	Predicted environmental concentration in sediment dry weight		mg/kg****

* The use of the indicated default values in the equations is recommended. Reasons for any deviations from these values should be given by the applicant.

** the characteristics of suspended matter are used in EqP calculations for sediment rather than the characteristics of bulk-sediment to reflect the concentration in the upper layer of the sediment, which is considered the major part of exposure for sediment dwelling organisms rather than via the deeper sediment layers.

*** The concentration in freshly deposited sediment is taken as the PEC for sediment. Therefore, the properties of suspended matter are used.

****If the PNECsed has to be expressed on a wet weight basis, the expression CONVsusp is omitted from the first equation

If the feed additive is not expected to degrade within a year (i.e. DT50 > 60 days at 12°C), the potential for residues to accumulate in sediment should be considered. In that case the PEC sed plateau should be used as calculated above.

3.3.5. Phase IIA PEC sediment calculation for marine and fresh water aquaculture

There are no advanced models accepted at EU level which can be suggested in this guidance for the refinement of the exposure for marine and freshwater aquaculture. In Phase I it is assumed that there is no retention in the system. In Phase II, for freshwater aquaculture, this could be considered as a further PEC refinement. An applicant could also present further assessment, using other modelling tools, more studies or relevant arguments provided that these models, studies and/or arguments are scientifically underpinned.

3.3.6. PNEC derivation based on minimum data requirements

The initial PNEC ($PNEC_I$) derivation is largely based on short-term toxicity tests. If for the same test species toxicity data of different quality are available as influenced for the experimental design of the study, those that are in line with OECD criteria for valid studies will be selected. If for the same species more than one valid and comparable (same endpoint and test duration) toxicity value is available, the geometric mean is used.

3.3.6.1. Terrestrial compartment

One nitrogen transformation test on soil microorganisms (28 days), one acute toxicity test on earthworms and one growth test in six different terrestrial plant species (at least two monocotyledonous and two dicotyledonous species) are required.

Tests required should be conducted according to OECD Guidelines 216 (Soil Microorganisms, Nitrogen Transformation Test (28 days)), 207 (Earthworm, Acute Toxicity Test) and 208 (Terrestrial Plants, Seedling Emergence and Seedling Growth Test).

The Phase IIA $PNEC_{I;soil}$ for soil organisms should be derived as described in Table 5, by selecting the lowest value:

Table 5: Ecotoxiticity studies required in Phase IIA to derive $PNEC_{I;soil}$

Study	Toxicity endpoint	AF	Remark
Nitrogen Transformation (28 days)	≤25% of control	1	Exposure 1X and 10X PEC_{max}
Terrestrial plants (14-21 d)	EC_{50}	100	The most sensitive endpoint (emergence, biomass or height of sprout) of all plant species tested
Earthworm acute (14 d)	LC_{50}	1,000	-

AF: assessment factor; EC_{50}: concentration of the additive causing effect in 50% of the population the most sensitive OECD endpoint; LC_{50}: concentration of the additive that kills 50% of the population

When a critical toxicity value (e.g. LC_{50},) concerns a 'larger than' value (i.e LC_{50}>5000 mg/kg), this value is used as a precautionary approach in the risk quotient.

When a sufficient number of appropriate chronic toxicity values (EC_{10} or NOEC values from long-term tests) for rooted plants (i.e. six plant species) and soil invertebrates are available, the Phase IIA $PNEC_I$ (which is assumed to be sufficiently conservative) may be superseded by a Phase IIC $PNEC_R$ (see Section 3.5.1).

3.3.6.2. Freshwater compartment (including sediment)

For feed additives to be used in terrestrial livestock animals and freshwater aquaculture, as a minimum Phase IIA dataset, one $L(E)C_{50}$ value each for a freshwater alga, a daphnid and a fish are required. For the assessment of the Phase IIA $PNEC_I$ for pelagic freshwater organisms the OECD Guidelines 201 (Freshwater Alga and Cyanobacteria, Growth Inhibition Test), 202 (*Daphnia* Acute Immobilization test) and 203 (Fish Acute Toxicity test) should be followed.

The Phase IIA $PNEC_{Isw}$ for pelagic water organisms should be derived as described in Table 6 by selecting the lowest value.

Table 6: Ecotoxicity studies required in Phase IIA to derive $PNEC_{I;sw}$

Study	Toxicity endpoint	AF	Remark
Algal growth inhibition	72-96 h E_rC_{50}	1,000	E_yC_{50} may be used if E_rC_{50} not reported
Daphnia immobilization	48-h EC_{50}	1,000	-
Fish acute toxicity	96-h LC_{50}	1,000	-

E_rC_{50} : the concentration of test substance which results in a 50 percent reduction in growth rate; E_yC_{50} : the concentration of the test substance with results in a 50% reduction of yield.

The assessment of acute toxicity tests considers the following statement of the OECD guidance document on the aquatic toxicity testing of difficult substances and mixtures (OECD, 2002): "It is important to note that an absence of acute toxic effects at the saturation concentration cannot be used as the basis for predicting no chronic toxicity at saturation or at lower concentrations."

A long-term test has to be carried out for substances showing no toxicity in short-term tests if the log K_{ow} > 3 (or a bioconcentration factor (BCF) > 100) and if the $PEC_{A\ sw}$ is > 1/100th of the water solubility. The long-term toxicity test should normally be a test on an invertebrate (preferred species *Daphnia;* OECD 211) to avoid unnecessary vertebrate testing.

According to REACH (ECHA, 2008) a log K_{oc} or log K_{ow} ≥ 3 for an organic chemical is used as a trigger value for sediment effect assessment. If this trigger is met, in Phase IIA the $PNEC_I$ of an organic feed additive for freshwater sediment-dwelling organisms will be derived on basis of the Phase IIA $PNEC_I$ for pelagic water organisms and the Equilibrium Partitioning (EqP) concept. The concept of EqP is based on the work of Di Toro et al. (1991).

According to the EqP concept, the PNEC for sediment organisms can be estimated as follows:

$$PNEC_{sed} = \frac{K_{susp-water}}{RHO_{susp}} \times PNEC_{surfacewater} \times 1000 \times CONV_{susp}$$

$$Ksusp\ water = Fwater\ susp + \left(Fsolid\ susp \times \frac{Kp\ susp}{1000} \times RHOsolid\right)$$

$$CONVsusp = \frac{RHO\ susp}{Fsolid\ susp * RHOsolid}$$

$$Kp_{susp} = Foc_{susp} \times K_{oc}$$

where:

Symbol	Parameter	Default Value*	Unit

Input			
$K_{susp\text{-}water}$	suspended matter**-water partition coefficient		m^3/m^3
RHO_{susp}	Bulk density of (wet) suspended matter***	1150	kg/m^3
RHO_{solid}	Bulk density of solids	2500	kg/m^3
$PNEC_{surfacewater}$	Predicted No Effect Concentration for aquatic organisms		µg/l
$CONV_{susp}$	Conversion factor for suspended matter concentrations: wwt to dwt		kg_{ww}/kg_{dw}
$Fwater_{susp}$	Volume fraction of water in suspended matter	0.9	m^3/m^3
1000	Conversion for litre to m^3		l/m^3
$Fsolid_{susp}$	Volume Fraction of solids in suspended matter	0.1	m^3/m^3
Kp_{susp}	Partition coefficient solids and water in suspended matter (v/w)		l/kg
K_{oc}	Organic carbon partition coefficient****		l/kg
Foc_{susp}	Weight fraction organic carbon in suspended solids	0.1	kg/kg
Output			
$PNEC_{sed;EqP}$	Predicted No Effect Concentration for sediment dwelling organisms		$\mu g/kg_{dw}$*****

* The use of the indicated default values in the equations is recommended. Reasons for any deviations from these values should be given by the applicant.
** the characteristics of suspended matter are used in EqP calculations for sediment rather than the characteristics of bulk-sediment to reflect the concentration in the upper layer of the sediment which is considered the major part of exposure for sediment dwelling organisms rather than via the deeper sediment layers.
*** The concentration in freshly deposited sediment is taken as the PEC for sediment. Therefore, the properties of suspended matter are used.
**** For a correct comparison the Koc value should be the same as used for the PEC calculation
***** When expressing $PNEC_{sed}$ on a wet weight basis, the expression CONVsusp is omitted from the first equation**.

EqP approach neglects sediment ingestion as a relevant uptake pathway, as it only represents transfer occurring through passive partitioning. According to REACH (ECHA, 2003; 2008), for chemicals with a log K_{ow} >5 an AF of 10 may be required to account for risks due to sediment ingestion.

The Phase IIA $PNEC_{I;sed;EqP}$ for sediment-dwelling organisms should be derived following Table 7.

Table 7: Procedure to derive Phase IIA $PNEC_{sed}$

Study	**Toxicity endpoint**	**AF**	**Remark**
Initial PNEC ($PNEC_I$) for pelagic water organisms and EqP approach	$PNEC_{I;sed;EqP}$	1 10	If the log Kow ≤ 5 If the log Kow > 5

EqP: equilibrium partitioning

When experimental chronic toxicity values (EC_{10} or NOEC values from long-term tests that assess sublethal endpoints) for sediment-dwelling organisms are available the Phase IIA $PNEC_{I;sed;EqP}$ (which is assumed to be sufficiently conservative) may be superseded by a Phase IIC $PNEC_{R;sed}$ (see Section 3.5.3)

3.3.6.3. Marine compartment

For feed additives used in mariculture, three marine sediment species have to be tested. At present, no internationally accepted, i.e. ISO or OECD, guidelines are available, except the 10-d ISO 16712 test for *Corophium volutator* (ISO, 2005). Several relevant guidelines are available from the American Society for Testing of Materials (ASTM) for toxicity in salt water systems which can be considered appropriate.

In the Phase IIA effect assessment the $PNEC_{I;sed}$ can be derived from sediment-spiked 10d toxicity tests with benthic organisms for which test protocols are available by applying an appropriate AF. In Phase IIC ($PNEC_{R;sed}$ derivation), chronic tests with these species will be considered.

An overview of the available sediment-spiked 10-d toxicity tests with marine/estuarine sediment-dwelling invertebrates is presented in Table 8. Note that nearly all test species mentioned in Table 8 concern crustaceans. In addition, a standard ASTM Guide for Conducting Renewal Microplate-Based

Life-Cycle Toxicity Tests with a Marine Meiobenthic Copepod (E2317-04) is available. This Copepod test, however, concerns a pore water test and not a sediment-spiked test.

Table 8: Overview of marine/estuarine benthic invertebrate test species for which protocols are available to conduct a 10-day sediment-spiked toxicity tests

Test species	Semi-chronic test guideline	Remark
Leptocheirus plumulosus (crustacean)	10d test; ASTM E1706 (ASTM, 2010a)	Occurs in estuarine habitats
Eohaustorius estuarius (crustacean)	10d test; US-EPA 1996 and ASTM E1367 (ASTM, 2010b)	Occurs in estuarine habitats
Ampelisca abdita (crustacean)	10d test; US-EPA 1996 and ASTM E1367 (ASTM, 2010b)	Occurs in marine habitats
Rhepoxynius abronius (crustacean)	10d test; US-EPA 1996 and ASTM E1367 (ASTM, 2010b)	Occurs in marine habitats
Corophium volutator (crustacean)	10d test; ISO 16712 (ISO, 2005)	Occurs in estuarine and marine habitats
Neanthes arenaceodentata (polychaete worm)	10d test; ASTM E1611 (ASTM, 2007)	Occurs in estuarine and marine habitats

ASTM: American Society for Testing of Materials; US EPA: United States Environmental Protection Agency

Based on available pesticides toxicity data (EFSA PPR Panel, 2015), there is no reason to assume that fresh water and marine/estuarine benthic invertebrates differ in their species sensitivity distribution for feed additives, although some taxonomic groups predominantly occur in freshwater habitats (e.g. Insecta) or marine/estuarine habitats (e.g. Polychaeta and Echinodermata). Assuming that species sensitivity distributions of benthic species do not differ substantially between freshwater and marine/estuarine habitats, also sediment-spiked 10-d protocol toxicity tests with freshwater invertebrates might be used if the AF for extrapolation is high enough. This approach is also adopted by the EFSA scientific opinion on the effect assessment for pesticides on sediment organisms (EFSA PPR Panel, 2015). An overview of the available sediment-spiked 10-d toxicity tests with freshwater sediment-dwelling invertebrates is presented in Table 9.

Table 9: Overview of freshwater benthic test invertebrates for which protocols are available to conduct a 10-day sediment-spiked toxicity tests

Test species	Semi-chronic test guideline	Remarks
Chironomus spp. (insect)	10d test; ASTM E1706 (ASTM, 2010a)	Insects are rarely found in marine/estuarine environments
Hexagonia spp. (insect)	10d test; ASTM E1706 (ASTM, 2010a)	Insects are rarely found in marine/estuarine environments
Hyalella azteca (crustacean)	10d test; ASTM E1706 (ASTM, 2010a)	Found in freshwater and estuarine environments
Diporeia spp. (crustacean)	10d test; ASTM E1706 (ASTM, 2010a)	-
Tubifex tubifex (oligochaete worm)	10d test; ASTM E1706 (ASTM, 2010a)	-

ASTM: American Society for Testing of Materials

The Phase IIA $PNEC_{I;sed}$ for sediment invertebrates in the marine environment should be derived following Table 10 by selecting the lowest toxicity value for the three benthic species

Table 10: Ecotoxiticity studies required in Phase IIA to derive $PNEC_{I;}sed$ for invertebrates in marine environment

Study	Toxicity endpoint	AF	Remark
Corophium volutator (ISO 16712)	10-d LC_{50}	1,000	Recommended marine species
Second marine/estuarine benthic species (Table 8)*	10-d LC_{50}	1,000	At least another taxonomic group than Crustacea is required in the data set
Third benthic marine/estuarine or freshwater species (Table 8 and 9)*	10-d LC_{50}	1,000	At least another taxonomic group than Crustacea is required in the data set

*If in the near future ISO and/or OECD guidelines for short-term toxicity tests with benthic species become available, these protocol tests are preferred.

In order to allow a correct comparison between the $PEC_{sed\ A}$ (the PEC_{sed} as assessed in Section 3.3.4. for sea cages) and initial $PNEC_{I;sed}$, the toxicity tests underlying the PNEC need to be normalised to the OC content of suspended solids used to derive the PEC sediment (i.e. 10% on dry weight basis) using the following equation:

$$NOEC\ or\ EC10_{standard} = NOEC\ orEC10_{experiment} \times \frac{Foc_{susp}}{Foc_{susp\ (experiment\ )}}$$

Alternatively the PEC and PNEC estimates can be expressed in terms of µg/g OC in dry sediment to allow a proper linking of exposure to effects.

When the adsorption is pH dependent it might also be appropriate to investigate whether the Koc value related to the pH of the sediment used in the toxicity test will significantly deviate from the Koc value used for the PEC calculation. If so, than the PNEC could be further normalised using the following equation.

$$NOEC\ or\ EC10_{standard,Koc\ normalised} = NOEC\ or\ EC10_{standard} \times \frac{Koc_{(pec)}}{Koc_{(experiment\ )}}$$

Note that when a sufficient number of chronic toxicity values (EC_{10} or NOEC values from long-term tests that assess sub-lethal endpoints) for sediment-dwelling invertebrates are available the Phase IIA $PNEC_{I;sed}$ (which is assumed to be sufficiently conservative) may be superseded by a Phase IIC $PNEC_{R;sed}$ (see Section 3.5.3).

3.3.7. Phase II A Risk assessment for secondary poisoning

If a substance has a log $K_{ow} \geq 3$ the risk for secondary poisoning (food web transfer) has to be assessed. For feed additives it might be appropriate to first consider if the safety assessment for the target species may also cover the assessment for secondary poisoning in non-target species or whether a separate assessment is needed. In this case, the methodology outlined in the *Guideline on environmental impact assessment for veterinary medicinal products in support of the VICH guidelines GL6 and GL38, Rev. 1* (EMA, 2016) should be followed.

3.3.8. Phase II A Risk characterisation

For the different compartments the calculated PEC_A's are compared with the initial PNEC ($PNEC_I$) derived; if the ratio of the PEC_A to the $PNEC_I$ is lower than 1, no further assessment is required. Otherwise, proceed with Phase IIB to refine the PECs when possible, or proceed to Phase IIC to refine the PNEC ($PNEC_R$) and recalculate the risk quotient (RQ) values. If PEC_A ground water is > 0.1 µg/L, proceed to Phase IIB.[6]

[6] If in the near future the specific protection goal for ground water organisms is adopted, the PEC_A ground water needs to be compared with the proper PNEC for ground water organisms.

3.3.9. Assessment of persistent, bioaccumulative and toxic substances

Feed additives that on the basis of the screening assessment in Phase I are considered to be potential PBT and/or vPvB substances need to be further assessed in Phase II with the PBT and vPvB criteria according to Section 1 of Annex XIII of the REACH Regulation.[7] These criteria together with the methodology in the REACH guidance on PBT/vPvB-assessment (Guidance on information requirements and chemical safety assessment Chapter R.11: PBT/vPvB Assessment and Chapters R.7a, R.7b, and R.7c on endpoints specific guidance) (ECHA, 2017) and the guideline on the assessment of persistent, bioaccumulative and toxic (PBT) or very persistent and very bioaccumulative (vPvB) substances in veterinary medicinal products (EMA/CVMP/ERA/52740/2012), should be considered.

Following the strategy outlined in these guidance documents a definitive assessment of P/vP, including assessment of any newly generated information, should be conducted first. Definitive assessment of P/vP should normally be based on degradation half-life data collected under adequate conditions for the relevant compartment(s) of exposure. For feed additives used in terrestrial and aquatic animals the most relevant compartment are soil and water/sediment systems, respectively.

If the substance is considered to fulfil the P and/or vP criterion, the PBT/vPvB assessment is continued by evaluation of the B/vB criterion including assessment of any newly generated additional information. Definitive assessment of B/vB should normally be based on measured data on bioconcentration in aquatic species. If such data is not yet available, it is recommended to conduct a bioaccumulation study in fish according to OECD 305.

If the substance is not identified as vPvB but considered to fulfil the P and B criteria, the PBT assessment is continued by evaluation of the T criterion based the standard aquatic toxicity studies described in section 3.3.6.2. Definitive assessment of T should be based on evaluation of the data for classification of the substance for human health hazards and/or on NOEC/EC_{10} values from long-term toxicity tests with aquatic organisms, including reproductive cycle tests when appropriate as indicated in section 3.5.2.2.

3.4. Phase II B to derive refined PEC estimates

Based on data not considered in Phase IIA, a more refined PEC can be calculated for each environmental compartment of concern. In ascertaining the refined PEC, account should be taken of:

- The potential degradation of the excreted active substance/metabolites of concern during normal manure processing practice and storage prior to its application to land;
- Other factors such as hydrolysis, photolysis, evaporation, etc.
- Use of more sophisticated models. The applicant is encouraged to check the Joint Research Centre (European Soil Data Centre) website for FOCUS models.[8]

3.4.1. PEC_B refinement for soil

3.4.1.1. Refinement based on degradation in manure

As a part of the Phase II assessment, data on degradation of the additive in manure may be submitted. Studies on degradation in manure should be performed according to the Guideline on determining the fate of veterinary medicinal products in manure (EMA, 2011).

As the storage capacity shows a large variation amonog the different EU Member States, it is recommended to set the storage capacity/time equal to the production period of the target animal up to three months, unless the number of cycles is more than four per year. In this case, the storage time is set equal to the period of the cycle. Indicative default values of storage time (days) were also published by EMA (2016).

[7] OJ L 396, 30.12.2006, p. 1

[8] FOCUS DG Sante, available online: https://esdac.jrc.ec.europa.eu/projects/focus-dg-sante

If degradation is to be considered in Phase II, the PEC_{manure} should be calculated for a storage time similar to one animal production cycle and, by doing so, the amount of manure is also set equal to the amount produced in that storage period, which fills the annual nitrogen quota of 170 kg N/ha. It is also necessary to consider that the animals could be given a feed additive at a particular period. If animals are given a feed additive at the beginning of the storage period there will be more time for the active ingredient to degrade than if they were given the additive at the end of the storage period. For this reason, the time for degradation of the active substance is taken to be half the storage time of the manure.

To calculate the $PEC_{soil\ B}$ by taking into account the degradation during storage, the following equations should be used:

$$PEC_{manure} = \frac{C_{add} \times FI_{total}}{N_{excreted}} \times e^{-kT_{st}/2}$$

$$k = \frac{\ln 2}{DT_{50}}$$

$$PEC_{soilB} = \frac{PEC_{manure} \times Q}{RHO_{soil} \times CONV_{area\ field} \times DEPTH_{field}}$$

where:

Symbol	Parameter	Default Value*	Unit
Input			
C_{add}	Concentration of the additive in feed		mg/kg complete feed
FI_{total}	Total feed intake (DM) per year		kg feed
$N_{excreted}$	Total N excretion per year		kg N
RHO_{soil}	Bulk density of (dry) soil	1,500	kg/m^3
$DEPTH_{field}$	Mixing depth with soil	0.05	m
$CONV_{area\ field}$	Conversion factor for the area of the agricultural field	10,000	m^2/ha
Q	Annual nitrogen load standard	170	kg N/ha
DT_{50}	Half-life of the additive in manure		day
K	Rate constant		
T_{st}	Length of time manure is stored		day
Intermediate results			
PEC_{manure}	Concentration of the additive (parent compound) in manure expressed per amount nitrogen		mg/kg N
Output			
$PEC_{soil\ B}$	Highest concentration of the additive (parent compound) in soil dry weight		mg/kg

* The use of the indicated default values in the equations is recommended. Reasons for any deviations from these values should be given by the applicant.

3.4.2. PEC refinement for groundwater, surface water and sediment and for additives used in livestock animals

The equations used in Phase IIA provide worst-case estimates of the exposure concentrations of the additive in pore water (see Section 2.6.2 and 3.3.2) and surface waters (see Section 3.3.3 and 3.3.4). If Risk Quotient (RQ) values for surface water organisms are > 1 and/or the PEC_{pw} is > 0.1 µg/l, more advanced models could be used to predict more realistic concentrations of the additive in deeper groundwater and surface waters.

More sophisticated models have been developed by the FOCUS (Forum for the Coordination of Pesticide Fate Models and Their Use) group. Justification for using these models is given in the EFSA

FEEDAP Panel (2007) opinion on the development of an approach for the environmental risk assessment of additives, products and substances used in animal feed.

The applicant could also present further assessment using other modelling tools, more studies or relevant arguments as to why exceeding the trigger value for groundwater or the RQ for aquatic organisms should not be considered a risk, provided that these models, studies and/or arguments are scientifically underpinned.

3.4.2.1. Groundwater

Groundwater calculations developed by FOCUS involve the simulation of the leaching behaviour of agrochemicals using a set of four models (PEARL, PELMO, PRZM and MACRO) in a series of up to nine geographic settings with various combinations of crops, soils and climate. Groundwater concentrations are estimated by determining the annual average concentrations in shallow groundwater (1m soil depth) for a period of 20 consecutive years, rank ordering the annual average values and then selecting the 80th percentile value (Metcalfe et al, 2016).

When using the FOCUS models, a simple first step of this assessment can be based on a realistic worst-case FOCUS scenario. For reasons given in the EFSA FEEDAP Panel (2007) opinion, it seems most appropriate to base such a leaching assessment on the FOCUS Okehampton scenario using PEARL.

In order to simplify the first step in the refined exposure assessment, calculations were performed with FOCUS_PEARL v3.0 applying a dose of 1 kg/ha on 3 October every year over a 20-year period. The dose was incorporated into the top 20 cm of soil. The crop was winter cereal. All substance properties except organic-matter/water distribution coefficient (K_{OM}) and DT_{50} were equal to the model substance D as defined by FOCUS. Runs were carried out with 90 K_{OM} - DT_{50} combinations covering FOCUS leaching concentrations ranging from 0.001 to about 100 µg/L. The results were fitted to a metamodel to be able to estimate leaching concentrations without running a FOCUS scenario (EFSA FEEDAP Panel, 2007). Based on this analysis, the following inequalities can be used for the first-tier leaching assessments of feed additives (see Table 11).

Table 11: Requirements for the K_{OM} as a function of the FOCUS leaching concentration

C_{FOCUS} (µg L^{-1})	Requirement for the K_{OM}
<0.001 - 0.01	K_{OM} > -5.9 + 9.1 DT_{50}
0.01 - <0.1	K_{OM} > -5.9 + 6.5 DT_{50}
≥0.1 – 1	K_{OM} > -5.9 + 3.8 DT_{50}
1 – 10	K_{OM} > -5.9 + 1.2 DT_{50}

K_{OM}= K_{oc}/1.7; DT_{50} : time to degrade half the concentration of the substance

The inequalities explain the requirement of K_{oc} and soil DT_{50} to define whether a substance is prone to leaching or not. The first two concentrations (C_{FOCUS}) identify compounds that do not leach to shallow groundwater. The third and fourth ones identify a possible leaching compound. In this last case, FOCUS models are needed to address the issue.

Note that these relationships are based on a dose of 1 kg/ha. In the event that the actual dose is substantially lower or higher, then a less or more stringent relationship should be used in proportion to the dose (e.g. when the dose is < 0.1 kg/ha, the relationship K_{OM} > -5.9 + 3.8 DT_{50} can be used to ensure the leaching concentrations is < 0.1 µg/L).

If it is not possible to exclude the likelihood that groundwater concentration is > 0.1 µg/L based on the metamodel, then it is necessary to run the PEARL model using the scenarios recommended in the EFSA FEEDAP Panel (2007) opinion. Table 12 indicates which scenarios have to be run for the specific target animals, taking into account the indicated considerations.

Table 12: Proposed FOCUS scenarios for $PEC_{B;gw}$ calculation of feed additives

Target animal	Bovine	Ovine	Swine	Avian
FOCUS GW	N: Jokioinen S: Sevilla, Piacenza	C: Okehampton S: Sevilla, Thiva	N: Jokioinen S: Piacenza	N: Jokioinen S: Piacenza

N: Northern/Scandinavian; C: Central; S: Southern/Mediterranean

Settings of the FOCUS model for groundwater

As explained above, manure application to arable land is most typically carried out in the early autumn. In order to standardise, the exposure assessments timing of application to soil is assumed to coincide with drilling of winter cereals (in the absence of pure grassland scenario) as these crops are typically grown throughout Europe and represent a significant input of manures on a total mass basis across Europe. The soil DT_{50} values should be the geometric mean values from the experimental data. In Section 3.3.1, guidance is given to select the most appropriate soil DT_{50} and K_{oc} values.

It is assumed that manure will be applied at a rate of 170 kg N/ha in one spreading event. As the input in FOCUS is expressed in kg/ha, the PEC_{soil} has to be converted to kg/ha before running the FOCUS model. Recommended input parameters on the application of FOCUS model is presented in the Appendix B.

3.4.2.2. Surface water

The surface water and sediment calculations developed by FOCUS include three progressively refined tiers of evaluation, ranging from initial spreadsheet-based evaluations of potential aquatic concentrations to more detailed mechanistic calculations of drift, runoff, erosion and field drainage loaded into a series of small water bodies. Additionally, a final Step 4 allows a detailed site-specific approach in case all previous Steps fail. The surface water and sediment calculations are performed using an overall calculation shell called SWASH which controls models that simulate runoff and erosion (PRZM), leaching to field drains (MACRO), spray drift (internal in SWASH) and finally aquatic fate in ditches, ponds and streams (TOXSWA). Those simulations provide detailed assessments of potential aquatic concentrations in a range of water body types in up to ten separate geographic and climatic settings.

Detailed explanations of the FOCUS models as well as the modelling scenarios, key assumptions, required modelling inputs and model outputs are provided in the respective FOCUS modelling reports (FOCUS, 2000; FOCUS, 2001) (EFSA FEEDAP Panel, 2007). The FOCUS surface water and groundwater models have been placed on a website (http://esdac.jrc.ec.europa.eu/projects/focus-dg-sante) where they can be freely downloaded.

Based on the EFSA FEEDAP Panel, 2007 opinion, the runoff and drainage scenarios given in Table 13 were identified as potential 'base-set' scenarios:

Table 13: Proposed FOCUS SWASH scenarios for $PEC_{sw\ B}$ and $PEC_{sed\ B;}$ calculation of feed additives

Target animal	Bovine	Ovine	Swine	Avian
FOCUS SW scenario (drainage)	D4	D6	D4, D3	D5, D3
FOCUS SW scenario (runoff)	R1, R3	R4	R1, R3	R1, R3

This selection covers not only the areas identified by FOCUS but also several areas in the Member States that joined the EU after May 2005 and is supported by a study carried within ERAPharm Project (Schneider et al., 2007).

If, when using FOCUS the OC fraction of the sediment on which the $PEC_{sed\ B}$ is based differs from that of the sediment used in toxicity tests, a normalisation of the PEC to a standard sediment is required (EFSA PPR Panel, 2015; see Section 9.3). Alternatively, the $PEC_{sed\ B}$ and the $PNEC_{R;sed}$ should be expressed in terms of µg/g OC in dry sediment to allow a proper linking of exposure to effects.

Settings of the FOCUS model for surface water

As proposed for groundwater, the application of manure to arable and grass land is considered to coincide with the drilling of cereals in autumn (in the absence of a pure grassland scenario). The soil DT_{50} values should be the geometric mean values from the experimental data. In section 3.3.1, guidance is given to select the most appropriate soil DT_{50} and K_{oc} values. In order to select the most appropriate application date, the FOCUS PAT (Pesticide Application Time) tool, part of the software package MACRO and PRZM, should be used. As a realistic worst case, it is assumed that manure will be applied at a rate of 170 kg N ha^{-1} in one spreading event. Without information on the degradation in a water/sediment, the degradation rate is set to zero. When needed, the PEC surface water could be further refined based on a water/sediment simulation study according to OECD TG 308. As mentioned for groundwater as the input in FOCUS is expressed in kg/ha, the PEC_{soil} has to be converted to kg/ha before running the FOCUS model. Recommended input parameters on the application of FOCUS model are presented in the Appendix C.

3.4.2.3. Interpretation of results from FOCUS

In FOCUS groundwater models the 80^{th} percentile annual average recharge concentrations leaving the top 1 m soil layer for a 20-year period is presented.

The results for surface water are presented as the maximum predicted PEC_{sw} and PEC_{sed} at the time of occurrence of the peak. The annual exposure profiles are presented graphically and PEC_{twa} concentrations for certain time-windows can be derived.

For further guidance to investigate leaching to groundwater under field conditions, the reader is referred to the FOCUS groundwater guidance (2014), and more details on FOCUS Surface Water models can be found in FOCUS (2015).

3.4.3. Phase II B Risk characterisation

For the different compartments the refined PEC_B's are compared with the initial PNEC ($PNEC_I$) derived. If the ratio of the PEC_B to the $PNEC_I$ is lower than 1, no further assessment is required. Otherwise, proceed with Phase II C to refine the PNECs when possible.

3.5. Phase II C to estimate refined PNEC ($PNEC_R$) values

For those additives where, following Phase IIA or Phase IIB assessment, an environmental risk cannot be excluded, further tests are needed to determine the chronic and more specific effects on appropriate microbial, plant and animal species. This additional information will allow the application of a lower AF.[9]

Suitable additional ecotoxicological tests are described in a number of publications, e.g., in OECD guidelines. Careful choice of such tests is necessary to ensure that they are appropriate to the situation in which the additive and/or its metabolites may be released and dispersed in the environment. The refinement of the effect assessment for soil ($PNEC_{R;soil}$) may be based on studies on the chronic effects on terrestrial invertebrates, additional studies on soil microflora and a number of relevant plant species.[10] The refinement of the effect assessment for water/sediment may be based on chronic toxicity tests on the most sensitive aquatic/benthic organisms identified in Phase IIA assessment. The refined PNEC ($PNEC_R$) derivation is largely based on chronic toxicity tests, including reproduction and/or developmental tests when suggested by previous indications. If for the same test species toxicity data of different quality are available (after normalisation in soil- and sediment-spiked test, see sections 3.3.6.1or 3.5.2.1) as influenced by the experimental design of the study, those that are in line with OECD criteria for valid studies will be selected. If for the same species more than one valid and comparable (same test duration and endpoint) toxicity value is available, the geometric mean is used.

The refinement of the risk assessment for secondary poisoning may be based on a bioaccumulation study in fish according to OECD 305.

[9] Regulation (EC) No 429/2008, OJ L 133 22.5.2008, p. 1.
[10] Regulation (EC) No 429/2008, OJ L 133 22.5.2008, p. 1.

3.5.1. Toxicity tests and $PNEC_{R\ soil}$ derivation: Terrestrial compartment

When for one or more of the taxonomic groups a risk has been identified, for these taxonomic groups, the PNEC can further be refined by the following chronic studies: the OECD Guidelines 216 (Soil Microorganisms, Nitrogen Transformation Test, 100 days), 208 (Terrestrial Plants, Growth Test, Additional species) and soil invertebrates (Earthworm Reproduction Test (220/222), springtail *Folsomia candida* (232) or the predatory mite *Hypoaspis aculeifer* (226)).

Field-collected soils used in ecotoxicological tests could differ in characteristics such as organic matter and clay content, soil pH and soil moisture content. The bioavailability of the test compound, and therefore the toxicity observed, could be influenced by those soil properties. This means that results from different test soils cannot be compared directly. If possible, data should be normalised using relationships that describe the bioavailability of chemicals in soils. If there is evidence that the bioavailability of the compound is related to the organic matrix, results are converted to a standard soil, which is defined as a soil with an organic matter content of 3.4% or an organic carbon content of 2.0 ±0.5% (since this OC fraction is also considered in calculating the PECsoil A or PECsoil B). Using an OC harmonised (2% on dry weight basis) PNECR estimate allows a proper linking of exposure to effects. Alternatively, toxicity estimates can be expressed in terms of µg/g OC in dry soil. The PNEC derived from such studies should then be compared with a PEC expressed in terms of µg/g OC in dry soil.

For the derivation of the $PNEC_{R\ soil}$ for terrestrial organisms, the same effect assessment is followed as performed for veterinary products (EMA, 2005), which means that separate assessment factors are applied to every taxonomic group. The lowest PNEC determines the $PNEC_{R\ soil}$ for the terrestrial compartment.

3.5.1.1. Terrestrial plants

At Phase IIA, the effect assessment for plants is based on the application of an assessment factor of 100 to the lowest EC_{50} value of six species (see Section 3.3.6.1). If a risk is identified in this lower tier, at Phase IIC the EC_{10} values from the most sensitive end point from all tested species should be used by applying an assessment factor of 10.

3.5.1.2. Terrestrial invertebrates

At phase IIA, the effect assessment for earthworms can be based on an acute toxicity study. The $PNEC_I$ is derived by applying an assessment factor of 1000 to the LC_{50} value.

If based on the acute earthworm toxicity test a risk cannot be excluded, at Phase IIC the chronic toxicity on earthworms (OECD guideline 220/222) and on a second soil invertebrate needs to be investigated (either springtail *Folsomia candida* (OECD guideline 232) or the predatory mite *Hypoaspis aculeifer* (OECD guideline 226). Note that the OECD guideline 222 requires the substance to be mixed into the soil and that clean manure is added to promote the reproduction of the earthworms. The test is not designed to study exposure via manure. The $PNEC_{R\ soil}$ is derived by applying an AF of 10 to the lowest EC_{10}/NOEC value. If there is evidence that the lowest EC_{10}/NOEC of the six terrestrial plants is at least one order of magnitude lower than the chronic EC_{10}/NOEC for earthworms, then no additional chronic toxicity test for a second invertebrate is needed.

3.5.1.3. Micro-organisms

The Soil Microorganisms, Nitrogen Transformation Test (OECD guideline 216) should be conducted at 1× and 10× the PEC. At Phase IIA, this study is conducted during a period of 28 days (see Section 3.3.6.1.). If, on day 28, differences between treated and untreated soils are ≥ 25%, at Phase IIC measurements have to be continued to a maximum of 100 days. When the difference in the rates of nitrate formation between the maximum PEC and control is ≤ 25% at any sampling after day 28 (considering sampling intervals of 14 d), the product can be evaluated as having no long-term influence on nitrogen transformation in soils.

3.5.1.4. $PNEC_R$ derivation for soil organisms

The Phase IIC $PNEC_R$ for soil organisms should be derived as indicated in Table 14, by selecting the lowest value

Table 14: Procedure to derive Phase IIC $PNEC_R$ for soil organisms

Study	Toxicity endpoint	AF	Remark
Terrestrial plants	14-21d EC_{10} (or NOEC)	10	Most sensitive end point of all tested species Section 3.5.1.1
Earthworm subacute/reproduction	56-d EC_{10} (or NOEC)	10	Section 3.5.1.2
Folsomia candida or *Hypoaspis aculeifer*	28-d EC_{10} (or NOEC) 14-d EC_{10} (or NOEC)	10	Section 3.5.1.2; not required if the EC_{10}/NOEC of most sensitive plant is at least 10 times lower than that of the earthworm
Nitrogen Transformation (100 days)	≤25% of control	1	Exposure 1x and 10x PEC_{max} Section 3.5.1.3

EC_{10}: the concentration of test substance which results in a 10 percent reduction of the effect tested; NOEC: no-observed-effect-concentration. It is usually the highest test concentration at which no toxic effects are observed;

If both the $PEC_{soil\ B}$ and refined $PNEC_{R;soil}$ estimates described above still trigger risks a further refinement of the effect assessment may be considered by conducting chronic laboratory toxicity tests with additional species (e.g. to allow the species sensitivity distribution (SSD) approach), by conducting a semi-field experiment and/or by advanced modelling approaches (e.g. EFSA PPR Panel, 2014a). It is advisable *a priori* to discuss the design of such advanced studies with the responsible regulatory authority. If at least one of the taxonomic groups mentioned in table 14 triggers a potential risk, and the most sensitive taxonomic group is at least an order of magnitude more sensitive, then an SSD approach focussing on this taxonomic group only is a logical step forward (see e.g. the EMA and EFSA PPR approach for terrestrial plants; EMA, 2017; EFSA PPR Panel, 2014b). If more taxonomical groups mentioned in Table 14 trigger potential risk, it may be appropriate to include several taxonomic groups in the SSD (see e.g. the REACH procedure in ECHA, 2008, Chapter R 10).

For plant $PNEC_R$ derivation on basis of the SSD approach, see the EMA CVMP Guideline on terrestrial plants (EMA, 2017).

3.5.2. Toxicity tests and refined PNEC derivation: Fresh water compartment

3.5.2.1. Freshwater pelagic and sediment-dwelling organisms

In order to refine the effect assessment for the freshwater compartment in case risks to pelagic organisms are triggered by Phase IIA or Phase IIB assessments, studies based on the OECD Guidelines 211 (*Daphnia magna* Reproduction), 210 (Fish, Early-life Stage) and the E_rC_{10} (or NOE_rC) derived from OECD Guideline 201 on algal Growth Inhibition are recommended. The latter study is already required in Phase IIA (for E_rC_{50} derivation). If from this study also a valid E_rC_{10} (or NOE_rC) can be derived, this value can be used without additional chronic tests (*Daphnia* and/or fish) to derive the $PNEC_{R;sw}$ when the E_rC_{50} for that alga is at least one order of magnitude more sensitive than the acute $L(E)C_{50}$ values for *Daphnia* and fish. If not, more standard test species chronic EC_{10} (or NOEC) values are required for $PNEC_{R;sw}$ derivation.

In addition, if risks of sediment exposure to benthic species is triggered in Phase IIA by a a $PNEC_{I;sed}$ derived on basis of the EqP approach (section 3.3.4), also the sediment-spiked Sediment-Water Chironomid Toxicity Test (OECD guideline 218) , the Sediment-Water *Lumbriculus* Toxicity Test (OECD Guideline 225) and a chronic EC_{10}/NOEC for a third benthic species are recommended. This latter species can be selected from test species mentioned in Table 19 (see below in Section 3.5.3.). Preferably the third benthic species is a freshwater species, but if an appropriate toxicity estimate is available for a marine/estuarine species this value may be used as well.

The composition of the sediment used for the tests depends on the requirements of the test species and should therefore follow that in the respective test methods. The use of artificial sediment is recommended. However, field collected sediment can also be used for the test as long as the properties of the sediment are described in detail.

The organic carbon content of sediment may influence bioavailability and consequently the toxicity of the test substance. Therefore, for comparison of sediment tests, the organic carbon content of the test sediment should be within a certain range. The OECD guideline 218 (Sediment-Water Chironomid Toxicity Test) and OECD guideline 225 (Sediment-Water Lumbriculus Toxicity Test Using Spiked Sediment) use sediment with an organic carbon content of 2 ± 0.5%. ASTM tests with benthic invertebrates usually use field-collected sediments that may vary in OC content. For the risk characterisation the toxicity estimates that underlie the $PNEC_{R;sed}$ should be normalised to the same organic carbon content that is used for the PEC calculation, i.e 10% in dry sediment, using the equation mentioned in section 3.3.6.3. If, when using FOCUS the OC fraction of the sediment on which the $PEC_{sed\ B}$ is based differs from that of the sediment used in toxicity tests, a normalisation of the PEC to a standard sediment is required (see Section 9.3 of EFSA PPR Panel, 2015). Alternatively, the $PEC_{sed\ B}$ and the $PNEC_{R;sed}$ should be expressed in terms of µg/g OC in dry sediment to allow a proper linking of exposure to effects. When the adsorption is pH dependent it might also be appropriate to investigate whether the Koc value related to the pH of the sediment used in the toxicity test does not deviate too much from the Koc value used for the PEC calculation. If so, than further adjustment could be considered as outlined in section 3.3.6.3.

3.5.2.2. Refined PNEC derivation for freshwater pelagic ($PNEC_{R;sw}$) and sediment ($PNEC_{R;sed}$) organisms

$PNEC_{sw}$ for pelagic freshwater organisms

The Phase IIC $PNEC_{R;sw}$ for pelagic water organisms should be derived as indicated in Table 15 and 16

Table 15: Endpoints to be used to derive the Phase IIC $PNEC_{R;sw}$ for pelagic organisms

Study	Toxicity endpoint	Remark
Algal growth inhibition	72-96 h E_rC_{10} or NOE_rC	E_yC_{10} or NOE_yC may be used if E_rC_{10} or NOE_rC not reported
Daphnia reproduction	21-d EC_{10} or NOEC	
Fish early life-cycle test	EC_{10} or NOEC	Duration of test dependent on test species

ErC_{10}: Concentration that reduces growth in 10%; EyC_{10}: Concentration that reduces the yield in 10%; NOEC: no observed effects concentration

Table 16: Assessment factors to apply to derive the Phase IIC $PNEC_{R;sw}$ for pelagic organisms based on the available ecotoxicity dataset

Available data	AF	Remark
One long-term EC_{10}/NOEC algae	100	An AF of 100 to the EC_{10} (NOEC) of the algae can only be applied if based on acute $L(E)C_{50}$ data there is evidence that algae are at least one order of magnitude more sensitive than *Daphnia* and fish.
Two long-term EC_{10}/NOECs (algae and *Daphnia or fish*)	50	Species tested should cover the most sensitive from the acute data set (Section 3.3.6.2). The lowest value should be used to derive the PNEC
Three long-term EC_{10}/NOECs	10	The lowest value should be used to derive the PNEC

EC_{10}: Concentration of the additive causing effect on 10% of the population; NOEC: no observed effects concentration

If both the $PEC_{sw\ B}$ and $PNEC_{R;sw}$ estimates described above still trigger risks a further refinement of the effect assessment may be considered by conducting chronic laboratory toxicity tests with additional species (e.g. to allow the SSD approach), by conducting a semi-field experiment and/or by advanced modelling approaches (e.g. TK-TD and population models; EFSA PPR 2014a). It is advisable *a priori* to discuss the design of such advanced studies with the responsible regulatory authority. The methods proposed by the ECHA Guidance (ECHA, 2008) and the EFSA Aquatic Guidance Document (EFSA PPR Panel, 2013) may be consulted for further guidance.

$PNEC_{R;sed}$ for freshwater sediment-dwelling organisms

The Phase IIC $PNEC_{R;sed}$ for freshwater sediment-dwelling organisms should be derived as indicated in Table 17 and 18. Note that in the Phase IIC $PNEC_{R;sed}$ derivation, sediment-spiked toxicity test are required only if the EqP approach based on the PNEC for freshwater pelagic organisms (either the Phase IIA $PNEC_{sw}$, but preferably the Phase IIC $PNEC_{R;sw}$) trigger a potential risk (see section 3.3.6.2).

Table 17: Endpoints to be used to derive the Phase IIC $PNEC_{R;sed}$ for freshwater sediment-dwelling organisms if the EqP approach triggers a potential risk

Study	Toxicity endpoint	Remark
Sediment-Water Chironomid Toxicity Test	28-d EC_{10} or NOEC	OECD 218
Sediment-Water *Lumbriculus* Toxicity Test	28-d EC_{10} or NOEC	OECD 225
Chronic test with other benthic freshwater or marine/ estuarine species	EC_{10} or NOEC	Table 19

EC_{10}: Concentration of the additive causing effect on 10% of the population; NOEC: no observed effects concentration

Table 18: Assessment factors to apply to derive the Phase IIC $PNEC_{R;sed}$ for sediment-dwelling freshwater organisms based on the available ecotoxicity dataset

Available data	AF	Remark
One long-term EC_{10}/NOEC (*Chironomus*)	100	Sediment-Water Chironomid Toxicity Test currently is a data requirement
Two long-term EC_{10}/NOEC (*Chironomus* and *Lumbriculus*)	50	-
Three long-term EC_{10}/NOECs (Table 17)	10	

EC_{10}: Concentration of the additive causing effect on 10% of the population; NOEC: no observed effects concentration

If both the $PEC_{sed\ B}$ and refined $PNEC_{R;sed}$ estimates for freshwater ecosystems described above still trigger risks a further refinement of the effect assessment may be considered by conducting chronic laboratory toxicity tests with additional sediment-dwelling species mentioned in Table 19 (e.g. to allow the SSD approach), by conducting a semi-field experiment and/or by advanced modelling approaches (e.g. toxico-kinetic / toxico-dinamic (TK-TD) and population models). The design of such advanced studies should be discussed *a priori* with the responsible regulatory authority. The methods proposed by the ECHA Guidance (ECHA, 2008), the EFSA Scientific Opinion on the effect assessment for pesticides on sediment organisms in edge-of-field surface water (EFSA PPR Panel, 2015) and Diepens et al. (2017) may be consulted for further guidance.

3.5.3. Toxicity tests and $PNEC_{Rsed}$ derivation: Marine compartment

In order to refine the effect assessment for the marine sediment compartment, long-term sediment-spiked tests with benthic invertebrates can be selected (see Table 19) informed by the results of Phase IIA $PNEC_{I;sed}$ assessment (Section 3.3.6.3). If in the near future other internationally approved ISO/OECD tests for sediment-spiked tests with marine/estuarine invertebrates become available these tests should be considered.

Table 19: Overview of freshwater and estuarine/marine benthic test species for which protocol tests are available for the conduct of chronic sediment-spiked toxicity tests

Test species	Long-term (chronic) test guideline	Remark
Chironomus spp. (insect)	28-65d tests; OECD 218 (OECD, 2004a) 44-100d life-cycle test; OECD 233 (OECD, 2010a)	Freshwater habitats
Hyalella azteca	(28-)42d test; US-EPA 1996; 2000 and	Freshwater and estuarine habitats

(crustacean)	ASTM E1706 (ASTM, 2010a)	
Lumbriculus variegatus (oligochaete worm)	28d test; OECD 225 (OECD, 2007)	Freshwater habitats
Caenorhabditis elegans (nematode worm)	4d test; ISO 10872 (ISO, 2010b)	Freshwater and soil habitats
Myriophyllum spicatum (vascular plant)	14d; OECD 239 (OECD, 2014)	Freshwater habitats
Myriophyllum aquaticum (vascular plant)	7d; ISO 16191(ISO, 2010a)	Freshwater habitats
Leptocheirus plumulosus (crustacean)	28d test; US-EPA 2001 and ASTM E1367 (ASTM, 2010b)	Estuarine habitats
Eohaustorius estuaries (crustacean)	28d test; US-EPA 1996	Estuarine habitats
Ampelisca abdita (crustacean)	28d test; US-EPA 1996	Marine habitats
Rhepoxynius abronius (crustacean)	28d test; US-EPA 1996	Marine habitats
Neanthes arenaceodentata (polychaete worm)	20-28d test; ASTM E1611 (ASTM, 2007)	Estuarine/marine habitats

The Phase IIC $PNEC_{R;sed}$ for sediment invertebrates in the marine environment is derived as indicated in Table 20 and 21.

Table 20: Endpoints to be used to derive the Phase IIC $PNEC_{R;sed}$ for sediment invertebrates in marine environment mentioned in Table 19.

Study	Toxicity endpoint	Remark
Marine/estuarine crustacean	EC_{10} or NOEC	
Second marine/estuarine benthic invertebrate	EC_{10} or NOEC	At least another taxonomic group than Crustacea is required in the data set
Third benthic marine/estuarine or freshwater invertebrate	EC_{10} or NOEC	At least another taxonomic group than Crustacea is required in the data set

If the full basic chronic data set (3 taxa) is not made available the $PNEC_{R;sed}$ for the marine environment, might be derived as indicated in Table 21, under the condition that the full short-term toxicity data set is available (Section 3.3.6.3)

Table 21: Assessment factors to apply to derive the Phase IIC $PNEC_{Rsed}$ for invertebrates in marine environment based on the available ecotoxicity dataset

Available data	AF	Remark
One long-term EC_{10}/NOEC	100	Species tested should cover the most sensitive species from the acute data set (Section 3.3.6.3)
Two long-term EC_{10}/NOEC values (different taxonomic groups)	50	Species tested should cover the most sensitive species from the acute data set (Section 3.3.6.3).
Three long-term EC_{10}/NOECs	10	Table 20

EC_{10}: Concentration of the additive causing effect on 10% of the population; NOEC: no observed effects concentration; AF: assessment factor

If both the $PEC_{sed\ B}$ and $PNEC_{R;sed}$ estimates for the marine environment described above still trigger risks a further refinement of the effect assessment may be considered by conducting chronic laboratory toxicity tests with additional sediment-dwelling species mentioned in Table 19 (e.g. to allow the SSD approach), by conducting a semi-field experiment and/or by advanced modelling approaches (e.g. TK-TD and population models; EFSA PPR Panel, 2014a). The design of such advanced studies should be discussed *a priori* with the responsible regulatory authority. The methods proposed by the

Technical Guidance for Deriving Environmental Quality Standards (EC, 2011) and Diepens et al. (2017) may be consulted for further guidance.

3.5.4. Phase II C Risk assessment for secondary poisoning

Using the methods indicated in Section 3.3.6, the QSAR estimate of the BCF value can be replaced by an experimental value determined in a study conducted according the OECD TG 305 to further refine the assessment of secondary poisoning when in phase IIB still a risk has been identified.

3.5.5. Phase II C Risk characterisation

For the different compartments the refined PNECs$_C$ are compared with the $PEC_{A/B}$ derived. If the ratio of the $PEC_{A/B}$ to the $PNEC_R$ is lower than 1, no further assessment is required. If not, a risk for the environment cannot be excluded and further mitigation measures should be considered.

4. Literature reviews

Reference can be made to published studies to support the safety of the additive for the environment. The additive (active substance(s)/agent(s)) in literature studies should be identical to that under application or, if not, should still allow conclusions on the additive under application to be made.

The list of relevant references included should be compiled in a reference management software and provided in .RIS format. Copies of the relevant papers should be provided. The applicant must ensure that terms and conditions asserted by any copyright holder of publications or information submitted to EFSA are fully satisfied. The applicant should consult with copyright licensing authorities (i.e. at national level) for guidance on purchasing copyright licenses to reproduce any publications provided to EFSA. The applicant remains solely responsible and liable for obtaining all necessary authorisations and rights to use, reproduce and share the publications provided to EFSA.

The EFSA guidance Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009 (EFSA, 2011) provides instructions on how to identify and select "scientific peer-reviewed open literature" and how to report it in a dossier. Even if the guidance was prepared to answer a specific question of the Regulation 1107/2009 for pesticide, the criteria described for the literature selection can be considered common to all chemicals.

This EFSA Guidance provides a definition of scientific peer-reviewed open literature and also provides instructions on how to identify, select and include scientific peer-reviewed open literature in a dossier and how to report the literature search and the selection process.

This EFSA Guidance is based on recognised best practices for evidence synthesis and is consistent with the fundamental principles of systematic review, to ensure methodological rigour and transparency, and to minimise bias in the identification and selection of scientific information in dossiers. The method for identifying and selecting scientific peer-reviewed open literature is based on three initial steps of the systematic review process, namely:

1. clarification of the objective of the review of the scientific literature and setting of the criteria for study relevance to the dossier;
2. searching for scientific literature; and
3. selection of relevant scientific literature for inclusion in the dossier.

The method is also consistent with a later step of the systematic review process, namely the clear and systematic reporting of the searching and study selection processes. This EFSA Guidance was developed by a working group that considered in detail how to pragmatically integrate best practices in evidence synthesis with the structure of existing Guidance documents to avoid unnecessarily increasing the effort needed to prepare and appraise dossiers. This EFSA Guidance is consistent with the existing EU and OECD Guidance documents that are widely used to assist the preparation of dossiers (SANCO, 2005; OECD, 2005, 2006).

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Abbreviations

AF	Assessment factor
BIOWIN	A wastewater treatment process simulator that ties together biological, chemical, and physical process models.
BCF	Bioconcentration Factor
C_{add}	Concentration of the additive (parent compound) in feed
C_{focus}	FOCUS leaching concentration (µg/L)
CF	Conversion factor (kg feed to kg carbon in faeces)
$CONV_{area\ field}$	Conversion factor for the area of the agricultural field
$CONV_{sed}$	Conversion factor for sediment concentrations: wwt to dwt
$DEPTH_{field}$	Mixing depth with soil
$DEPTH_{sed}$	Mixing depth in sediment
DF	Dilution Factor
DT_{50}	Half-life of additive
DT_{90}	Time to degradation of 90% of original concentration of the compound in the tested soils
EC_{50}	The concentration of a test substance which results in 50% of the test organisms being adversely affected, i.e., both mortality and sub-lethal effects
E_rC_{50}	The concentration of a test substance which results in a 50% of inhibition of algal growth rate
EqP	Equilibrium partitioning
ERC	Ecologically relevant type of concentration
Fa	Fraction of the dose considered to be active
$Fair_{soil}$	Fraction air in soil
Fd	Fraction of additive (parent compound) degraded in 1 year
FI_{total}	Total feed intake (DM) per year
Flow	Water flow rate through the system
Foc_{sed}	Weight fraction organic carbon in sediment
Foc_{soil}	Weight fraction organic carbon in soil
FOCUS	The FOrum for Co-ordination of pesticide fate models and their USe
FR	Feed Ration
F_{rs}	Fraction remaining in soil after time $T_{interval\ spreading}$
$F_{solid\ sed}$	Volume Fraction of solids in sediment
$F_{solid\ soil}$	Fraction solids in soil
$F_{water\ sed}$	Volume fraction of water in sediment
$F_{water\text{-}soil}$	Fraction water in soil
k	Rate constant
$K_{air\text{-}water}$	Partition coefficient air and water in soil
K_d	Sorption/desorption coefficient
k_{dep}	Maximum deposition rate of faeces

K_{oc}	Sorption/desorption coefficient, normalized to organic carbon content
K_{OM}	Organic-matter/water distribution coefficient (L/kg). It corresponds to K_{oc} /1.724
K_{ow}	n-Octanol/water partitioning coefficient
Kp_{sed}	Partition coefficient solids and water in sediment (v/w)
Kp_{soil}	Partition coefficient solids and water in soil (v/w)
$K_{sed\text{-}water}$	Sediment-water partition coefficient
$K_{soil\text{-}water}$	Partition coefficient solids and water in soil (v/v)
LC_{50}	The concentration of a test substance which results in a 50% mortality of the test species
MOLW	Molar mass
$N_{excreted}$	Total N excretion per year
NOEC	No-observed effect concentration, i.e., the test concentration at which no adverse effect occurs
$N_{spreading}$	Number of spreading events
OC	Organic carbon
OECD	Organization for Economic Co-operation and Development
PAT	Pesticide Application Timer
PBT	Persistent, bioaccumulative and toxic substance
PC_{faeces}	Concentration of the additive (parent compound) in faeces
PEC_{faeces}	Predicted concentration of the additive (parent compound) in faeces
$PEC_{fw\ sed}$	Concentration of the additive (parent compound) in fresh water sediment
PEC_{manure}	Concentration of the additive (parent compound) in manure expressed per amount nitrogen
$PEC_{porewater}$	Concentration of the additive (parent compound) in porewater
PEC_{sed}	Concentration of additive (parent compound) in sediment
$PEC_{sed\ refined}$	Refined concentration of the additive (parent compound) in sediment
PEC_{soil}	Concentration of the additive (parent compound) in soil
$PEC_{soil\ 1\ year}$	Concentration of the additive (parent compound) 1 year after spreading
$PEC_{soil\ initial}$	Concentration of the additive (parent compound) immediately after spreading
$PEC_{soil\ plateau}$	PEC_{soil} at plateau concentration
$PEC_{soil\ refined}$	Refined concentration of the additive (parent compound) in soil
$PEC_{soil\ single\text{-}event}$	Concentration of the additive (parent compound) in soil immediately after spreading
PEC_{sw}	Concentration of the additive (parent compound) in surface water
$PEC_{max\ sw}$	Highest initial concentration of additive (parent compound) in surface water
$PNEC_{sed}$	Predicted No Effect Concentration for sediment dwelling organisms
$PNEC_{sw}$	Predicted No Effect Concentration for aquatic organisms
PRZM	Pesticide Root Zone Model
Q	Annual nitrogen load standard
QPS	Qualified Presumption of Safety approach for risk assessment of microbials

R	Gas constant
REACH	Regulation of the European Union, adopted to improve the protection of human health and the environment from the risks that can be posed by chemicals, while enhancing the competitiveness of the EU chemicals industry. It also promotes alternative methods for the hazard assessment of substances in order to reduce the number of tests on animals.
RHO_{susp}	Bulk density of (wet) suspended matter
RHO_{soil}	Bulk density of fresh wet soil
RHO_{solid}	Bulk density of solids in soil or sediment
RQ	Risk quotient
SOL	Water solubility
SSD	Species sensitivity distribution approach
SWASH	Surface Water Scenarios Help
TEMP	Temperature at air-water interface
$T_{interval\ spreading}$	Time between spreading events
TK-TD	toxicokinetic-toxicodynamic models
TOXSWA	TOXic substances in Surface Waters
$T_{production}$	Number of production days
T_{st}	Length of time manure is stored
VICH	Veterinary international Cooperation on Harmonisation
VP	Vapour pressure
vPvB	Very persistent and very bioaccumulative substance
wt	Weight

Glossary

Appendix A – Specific protection goal options and associated exposure assessment goal options for environmental risk assessments of feed additives

A.1. General protection goals

A.1.1. Introduction

Feed additives are subject to an environmental risk assessment (ERA) before they can be approved for placing on the market. The first step of an ERA is to establish the context for the assessment by identifying which ecosystems/habitats of the environment potentially become exposed by feed additives, and which components of these ecosystems/habitats (e.g. species, ecosystem services) are valued by civil society and/or protected by relevant laws and policies. In Regulation (EC) No 1831/2003 on additives for use in animal nutrition (EC, 2003) the following general statements can be found to protect the environment:

- In order to protect ....the **environment**, feed additives should undergo a **safety assessment** through a Community procedure before being placed on the market...
- Action by the Community relating to....the environment should be based on the **precautionary principle**
- It is necessary to introduce....a post-market monitoring plan in order to trace and **identify any direct or indirect, immediate, delayed, or unforeseen effect** resulting from the use of feed additives on.....the environment.....
- The purpose of this Regulation is to establish a Community procedure for authorising the placing on the market and use of feed additives ....in order to provide the basis for the assurance of a **high level of protection** of.....the environment
- The feed additive shall not have an **adverse effect** on...the environment

Since the current ERA for feed additives aims to harmonise with the ERA procedures for veterinary medicinal products (VMPs) it is important to also consider the general statements on environmental protection in CVMP/VICH (2005). In this document the following statements on protection goals can be found:

- The **overall target** is the **protection of ecosystems**
- The aim of the guidance is to **assess** the **potential** for VMPs **to affect non-target species** in the environment, including **both aquatic and terrestrial species**
- The taxonomic levels tested are intended to serve as surrogates or indicators for the range of species in the environment
- **Impacts of greatest potential concern** are usually those at **community and ecosystem function levels**, with the **aim** being **to protect most of species**
- There may be a need to **distinguish between local and landscape level**
- Issues associated with **cumulative impact** of some VMPs may be **appropriate at the landscape level**
- Residues are generally assumed to be uniformly distributed in the environment, even though distribution may be patchy

A.1.2. Environmental compartments and organisms to be protected

From the information presented in Regulation (EC) No 1831/2003 and its implementing rules, the Technical Guidance for ERA of feed additives (EFSA, 2008a) and discussions with risk managers it is clear that at least an ERA should be conducted for (1) non-target organisms in agricultural **soils that receive animal manure/slurry** contaminated with feed additives, (2) non-target organisms in the

water and sediment compartment of surface waters subject to input of feed additives via drainage and run-off from agricultural fields, or via land-based fish farms, (3) the non-target organisms in the **sediment compartment** under fish cages in the marine environment, and (4) **the quality of deeper groundwater** as influenced by leaching of feed additives from soil.

Considering the quality of deeper groundwater it is understood that the trigger value for groundwater concerns the groundwater quality standard for pesticides of 0.1 µg/L. Although not explicitly mentioned in Regulation (EC) No 1831/2003 possible specific protection goals (SPGs) for typical groundwater communities and dung fauna were also explored by the working group although no typical dung fauna for poultry dung/manure could be identified.

While feed additives might have a positive or negative influence on air quality (methane emission, N_2O) this is considered beyond the scope of this technical guidance, since environmental risk assessment on this topic is not addressed in Regulation (EC) No 1831/2003 nor requested by risk managers.

Direct or indirect, immediate, delayed, or unforeseen effects of feed additives and their metabolites on non-target organisms in soil, surface water and sediment need to be identified to ensure a high level of protection. This suggests that also impacts of long-term exposures should be assessed (need for chronic effect assessment procedure, or an appropriate extrapolation of results of an acute effect assessment procedure).

In the previous Technical Guidance that needs to be updated reference is made to a stepped ERA approach based on Risk Quotients (RQs) = PEC/PNEC values. The use of PNECs in the effects assessment suggests that no adverse effects on plant and animal species or processes performed by microbes are allowed. Although not explicitly mentioned, the protection of non-target plants and animal species likely concerns the population level and that of microbes the functional group level. In defining specific protection goal (SPG) options this should be made more explicit.

According to Regulation (EC) No 1831/2003 the ERA for feed additives and their metabolites should be based on the precautionary principle. This can be interpreted as follows: In the absence of relevant and reliable data the ERA should be based on worst-case assumptions, while this can be relaxed if these data become available.

A.2. Deriving Specific Protection Goals (SPGs)

Policy protection goals as described in Regulation (EC) No 1831/2003 are too generic and vague to be directly used in ERA schemes for feed additives. Terms like "high level of protection" and "risks of adverse effects" need to be operationalised. EFSA has developed a procedure to operationalise generic protection goals and to define Specific Protection Goals (SPG) for ERA schemes and regulatory decision making by using the Ecosystem Services Concept (EFSA PPR Panel, 2010; EFSA SC, 2016a). Ecosystem services are the benefits people obtain from ecosystems. They include provisioning services such as food and water; regulating services such as flood and disease control; cultural services such as spiritual, recreational, and cultural benefits; and supporting services such as nutrient cycling that maintain the conditions for life on Earth (Millennium Ecosystem Assessment, 2005).

EFSA's ecosystem service-based framework to define SPGs follows sequential steps:

1. Identifying ecosystems/habitats potentially impacted by the regulated product or agent
2. Identifying relevant ecosystem services potentially impacted by the exposure to the regulated product/agent in these ecosystems/habitats

3. Identifying service-providing units (SPUs), the structural and functional components of biodiversity that provide or support these ecosystem services
4. Specifying the level of protection of these SPUs by using the following dimensions: (a) **ecological entity** of the SPU to be protected , e.g. individual, population, functional group, (b) the **attribute to protect** , e.g. survival, abundance, biomass, processes, (c) the **maximum tolerable impact** , e.g. negligible - <10%; small- >10% < 30%; medium->30% <60%; large >60%, (d) **temporal scale** of tolerable effect, e.g. < 1 day; days, weeks, months, (e) **spatial scale** of tolerable effect, e.g. field, edge-of-field, watershed/landscape)
5. Evaluation whether **standard test species and endpoints** already adopted, or mentioned as data requirements, in regulatory frameworks can be **linked to the SPGs options** identified
6. **Linking of SPG options** developed for specific SPU groups to **vulnerable species** within this SPU (or grouped SPUs). This is important for the development of a tiered ERA scheme that overall is protective for all field species within SPU groups covered by the SPG and that are potentially at risk. Vulnerability of a species is determined by (i) the chance to become exposed to the feed additive (and/or its major metabolites), (ii) the intrinsic sensitivity to the chemicals of concern, (iii) the potential for ecological recovery, and (iv) species-traits that make the species susceptible to indirect effects. If in ERA schemes the aim is to accept negligible population-level effects only (ecological threshold option), the chance to become exposed and the intrinsic sensitivity are the main drivers for the risk assessment. If in ERA schemes some population-level effect are locally accepted under the condition that ecological recovery takes place (ecological recovery option) then all aspects of vulnerability should be considered (see also EFSA SC, 2016b)
7. Identifying the ecotoxicologically relevant type of concentration (ERC) to select as 'C' in the effect estimates such as the laboratory toxicity data to derive a PNEC and the field exposure estimates or PECs (e.g. for soil or sediment organisms the total concentration of the substance in dry soil or dry sediment or the freely dissolved fraction in pore water of soil or sediment)

A.3. SPG options for feed additives and aquatic SPUs (including those of groundwater ecosystems)

A.3.1. SPG options for aquatic ecosystems (water and sediment organisms)

Building on the experience of using the EFSA approach in defining SPGs for aquatic organisms and plant protection products (e.g. EFSA PPR Panel, 2010; EFSA PPR Panel, 2013; EFSA PPR Panel, 2015) the SPU organism groups mentioned below (Tables 1) might be useful for ERA of feed additives. In this table relevant SPU organism groups and related standard test species frequently used in aquatic ERA are mentioned, as well as the standard test species required for feed additives in the EFSA FEEDAP 2008 ERA guidance document.

Coccidiostats used as feed additives have a specific mode of action that may impact Protozoa. For this reason Protozoa are included as a relevant group of SPU organisms.

For persistent mobile substances, there is a concern that they may affect typical ground water species. These species generally have a longer life span than taxonomically related aquatic species that dwell in surface waters. In addition, if they are impacted, the decline in population density will last longer because of their poor ability to recolonize impacted groundwater habitats. In other words, typical groundwater species may be more vulnerable than taxonomically related species in surface water.

According to EMA (2017) and Kolar and Finizio (2017), and literature cited, the largely unrecognised biodiversity in groundwater ecosystems needs more attention in ERA and they propose that the protection of groundwater organisms should be a compulsory part of the overall environmental risk assessment for contaminants, including pharmaceuticals and feed additives. Important groundwater habitats can be found on hypogean karst (fractures, channels, caves) and alluvial gravel interstitial systems. Since spring habitats (the transition between groundwater and surface water) are fed by groundwater, the typical organisms living there also deserve protection. An important element to be considered for the ERA of groundwater ecosystems is prolonged exposure and the need to conduct chronic assessments. The components of biodiversity of groundwater ecosystems that need special attention are flatworms, annelids, molluscs, arthropods (e.g. *Niphargus* ssp.) and amphibians (e.g. *Proteus anguinus*). Currently no specific standard tests are developed for typical groundwater fauna, so that the OECD tests developed for typical freshwater invertebrates and vertebrates need to be considered as surrogate test species.

Table 1: *Overview of relevant aquatic SPU organisms, examples of related standard test species and current (2017) basic data requirements in the EFSA FEEDAP 2008 ERA guidance for feed additives*

SPU-Organism group	Examples of standard test species /assays	Phase II data requirements Feed additives
Aquatic microbes	OECD test on inhibition of anaerobic bacteria in sludge or sediment; ISO test on inhibition of nitrification in activated sludge	No
Aquatic Protozoa	Currently no official OECD Test Guideline available (the freshwater protozoan *Tetrahymena pyriformis* and the marine protozoan *Uronema marinum* may be good candidates for guideline development)	No
Algae	OECD tests with algae (e.g. *Pseudokirchneriella subcapitata*)	Yes
Aquatic macrophytes	OECD tests with *Lemna* sp. and *Myriophyllum spicatum*	No
Aquatic arthropods	OECD tests with *Daphnia* sp. and *Chironomus* sp.; ASTM test with *Hyalella azteca, Diporeia* spp., *Leptocheirus plumulosus, Eohaustorius estuarius, Ampelisca abdita, Rhepoxynius abronius* and *Hexagonis* spp.; ISO test with *Corophium volutator*	Yes, for freshwater ecosystems *Daphnia magna* and a sediment dwelling organism (e.g. *Chironomus*) Yes for marine sediment dwelling taxa (e.g. *Leptocheirus*, *Ampelisca*, *Rhepoxynius* and *Corophium*)
Other invertebrates	OECD test with *Lumbriculus variegatus*; ISO test with *Caenorhabditis elegans*; ASTM test with *Neanthes arenaceodentata*	Yes, for freshwater ecosystems a sediment dwelling organism (e.g. *Lumbriculus or Caenorhabditis*) Yes for marine sediment dwelling taxa (e.g. *Neanthes*)
Aquatic vertebrates	OECD test with *Oncorhynchus mykiss;* ASTM test with *Rana pipiens*	Yes for freshwater fish

Note that in the data requirements underlying the FEEDAP 2008 guidance, standard tests with aquatic microbes, aquatic protozoans and aquatic macrophytes, currently are not mentioned. Standard tests with an alga, *Daphnia magna* and a sediment organism (aquatic invertebrates) and fish (aquatic vertebrate) are required. It is uncertain, but assumed, that for exposure to feed additives these standard test species sufficiently cover the SPG for microbes, protozoans and aquatic vascular plants.

According to EFSA PPR (2010) and EFSA SC (2016a), overall most non-target organisms need to be protected at the population-level, except microbes and vertebrates. The selected ecological entity for microbes is the functional group and the attribute to assess are processes. Also note that it currently is almost impossible to assess chemical effects on microbes at the population-level. The selected ecological entity for vertebrates is set at the individual (acute toxicity) to population (chronic toxicity) level, since suffering of vertebrates due to exposure to regulated agents generally is not accepted by risk managers and the public at large. All options presented below assume that when protecting the selected SPU-key organism groups in aquatic habitats nearby the site of application, this also will guarantee a high level of protection in more remote aquatic habitats where the exposure to feed additives (and their major metabolites) most likely will be lower than nearby the site of application.

Three SPG options for feed additives and pelagic and benthic aquatic organisms are presented below (Tables 2-4), viz.: (A) the high margin of safety option, (B) the ecological threshold option, and (C), the ecological recovery option.

A.3.1.1. The high margin of safety option for pelagic and benthic aquatic organisms

The 'high margin of safety option' (see Table 2) assumes that an extra margin of safety should be used when assessing the risks of individual (types of) feed additives, since aquatic organisms may become exposed simultaneously to different types of feed additives that are assessed separately, or the presence of endangered species in the aquatic habitats of concern may require a precautionary approach (see also EFSA SC, 2016c). The extra margin of safety may be achieved by applying an extra Assessment Factor to the PNEC derived for the substance(s) under evaluation (here provisionally placed under the SPG dimension Magnitude of tolerable effect). Taking into account the vulnerability of groundwater fauna and the lack of standard test protocols for groundwater invertebrates and vertebrates, EMA (2017) and Kolar & Finizio (2017) propose to adopt a precautionary approach by applying an extra AF of 10 to the PNEC derived for typical freshwater test species.

Table 2: *Overview of proposed aquatic SPU organisms and their SPG dimensions for the 'high margin of safety option'*

SPU-Organism group	Ecological entity	Attribute	Magnitude of tolerable effect	Temporal scale	Spatial scale
Aquatic microbes	Functional group	Processes	Negligible + extra AF	< days	(Near) site of application
Aquatic Protozoa	Functional group or population?	Processes or abundance?	Negligible + extra AF	< days	(Near) site of application
Algae	Population	Abundance/ biomass	Negligible + extra AF	< days	(Near) site of application
Aquatic macrophytes	Population	Abundance/ biomass	Negligible + extra AF	< days	(Near) site of application
Aquatic arthropods	Population	Abundance/ biomass	Negligible + extra AF	< days	(Near) site of application
Other invertebrates (e.g. worms and molluscs)	Population	Abundance/ biomass	Negligible + extra AF	< days	(Near) site of application
Aquatic vertebrates (e.g. fish and amphibians)	Individual	Survival	Negligible + extra AF	< days	(Near) site of application
	Population	Abundance/ biomass			

A.3.1.2. The ecological threshold option for pelagic and benthic aquatic organisms

This 'ecological threshold option' (Table 3) assumes that by only allowing negligible effects of exposure to a specific (type of) feed additive, the SPU-key organism groups will be sufficiently protected also in case of simultaneous exposure to different types of feed additives. Since the magnitude of tolerable effect is set at negligible for this option, the ecological threshold option seems to be the option that up till now is used by calculating the PEC/PNEC ratio on basis of the most sensitive (standard) test species.

Table 3: *Overview of proposed aquatic SPU organisms and their SPG dimensions for the 'ecological threshold option'*

SPU-Organism group	Ecological entity	Attribute	Magnitude of tolerable effect	Temporal scale	Spatial scale
Aquatic microbes	Functional group	Processes	Negligible	< days	(Near) site of application
Aquatic Protozoa	Functional group or population?	Processes or abundance?	Negligible	< days	(Near) site of application
Algae	Population	Abundance/ biomass	Negligible	< days	(Near) site of application
Aquatic macrophytes	Population	Abundance/ biomass	Negligible	< days	(Near) site of application
Aquatic arthropods	Population	Abundance/ biomass	Negligible	< days	(Near) site of application
Other invertebrates (*e.g. worms and molluscs*)	Population	Abundance/ biomass	Negligible	< days	(Near) site of application
Aquatic vertebrates (*e.g. fish and amphibians*)	Individual	Survival	Negligible	< days	(Near) site of application
	Population	Abundance/ biomass			

A.3.1.3. The ecological recovery option for pelagic and benthic aquatic organisms

This 'ecological recovery option' (Table 4) allows a local but temporal effect on processes by aquatic microbes, and on population structure of aquatic algae and aquatic invertebrates, as long as the permissible direct effects do not result in unacceptable indirect effects. Note that when selecting this option the ERA scheme should be protective as well for vulnerable field populations within the SPU-key organism groups. This may not be feasible if organisms at stake are potentially sensitive, have a long and complex life-cycle and a limited dispersal capacity. In addition, when selecting this option the ERA may need to be conducted at the local and landscape level if external recovery processes and 'action at a distance' play a prominent role, which can be assumed for mobile aquatic invertebrates and fish (see EFSA SC, 2016b).

Table 4: *Overview of proposed aquatic SPU organisms and their SPG dimensions for the 'ecological recovery option'*

SPU-Organism group	Ecological entity	Attribute	Magnitude of effect	Temporal scale	Spatial scale
Aquatic microbes	Functional group	Processes	Small	Months	(Near) site of application
			Medium	Weeks	
			Large	Days	
Aquatic Protozoa	Functional group or population?	Processes or abundance?	Small	Months	(Near) site of application
			Medium	Weeks	
			Large	Days	
Algae	Population	Abundance/	Small	Months	(Near) site of

		biomass	Medium	Weeks	application
			Large	Days	
Aquatic macrophytes	Population	Abundance/ biomass	Small	Months	(Near) site of application
			Medium	Weeks	
			Large	Days	
Aquatic arthropods	Population	Abundance/ biomass	Small	Months	(Near) site of application and possibly watershed for mobile species
			Medium	Weeks	
			Large	Days	
Other invertebrates (*e.g. worms and molluscs*)	Population	Abundance/ biomass	Small	Months	(Near) site of application and possibly watershed for mobile species
			Medium	Weeks	
			Large	Days	
Aquatic vertebrates (*e.g. fish and amphibians*)	Individual	Survival	Negligible	< days	(Near) site of application
	Population	Abundance/ biomass			

A.3.2. Selected SPG option for aquatic ecosystems (water and sediment organisms)

After the description of the SPG options by the working group, they were presented to the FEEDAP Panel and risk managers of the European Commission. Risk managers indicated that they require more time to evaluate the proposed SPGs and the procedure to derive them, as well as the possible consequences (cost-benefit analysis) for placing feed additives on the European market. Based on the oral comments received it was decided to select the 'Ecological Threshold Option' as SPG for water and sediment organisms. This option is most in line with the ERA schemes developed for feed additives in the old Technical Guidance. Since risk managers did not (yet) request developing ERA decision schemes for exposure of typical groundwater organisms to feed additives, the protection goal of deeper groundwater remains for the time being the ground water quality standard of 0.1 µg/L (Directive 2006/118/EC).[11]

A.4. Example of SPG options for feed additives and terrestrial SPUs

A.4.1. Soil organisms

Similar SPG options as presented for ERA of feed additives and aquatic sediment-inhabiting organisms can be used for soil organisms exposed to feed additives in agricultural fields. Again, the three SPG options mentioned below for soil organisms have the same SPU-key group organisms. In Table 5 relevant soil SPU organism groups and related standard test species frequently used in ERA for soil organisms are mentioned, as well as the current standard test species required for feed additives. Since coccidiostats are an important group of feed additives, Protozoa are included as a relevant group of SPU organisms.

Note that for feed additives the basic data requirements underlying the EFSA FEEDAP 2008 ERA guidance document comprise studies with three plant species. It therefore seems that for the agricultural soil compartment the provisioning services of (crop) plants have a high priority.

Furthermore, in these data requirements standard tests with soil arthropods (e.g. predatory mites and collembolans), other soil invertebrates (e.g. nematodes, molluscs and enchytraeids) and soil vertebrates (e.g. mole) were not mentioned. It apparently was assumed that the required standard

[11] Directive 2006/118/EC of the European Parliament and of the Council of 12 December 2006 on the protection of groundwater against pollution and deterioration. OJ 27.12.06, L 372/20.

tests for microbes, terrestrial plants and earthworms sufficiently cover the SPG for these SPU groups (Table 5). It may be argued that potential riks of feed additives to typical soil vertebrates (e.g. mole) is already covered by the risk assessment of livestock animals.

Table 5: *Overview of relevant soil SPU organisms, examples of related standard test species and basic data requirements in the EFSA FEEDAP 2008 ERA guidance document*

SPU-Organism group	Examples of standard test species /assays	Phase II data requirements Feed additives
Soil microbes	OECD nitogen transformation test; ISO test on spore germination of mycorrhizal fungi	Yes
Soil Protozoa	?	No
Terrestrial plants	OECD tests on terrestrial plants (seedling emergence and growth; vegetative vigour)	Yes, studies with three plant species
Earthworms	OECD/ISO earthworm tests (*Eisenia fetida*/ *Eisenia andrei*)	Yes
Soil arthropods	OECD/ISO predatory mite (*Hypoaspis aculeifer*) and collembolan (*Folsomia*) test	No
Other soil invertebrates	ISO test with *Caenorhabditis elegans*	No
Soil vertebrates	?	No

A.4.1.1. The high margin of safety option for soil organisms

The 'high margin of safety option' (see Table 6) assumes that an extra AF should be used when assessing the magnitude of tolerable effects for individual (types of) feed additives, since soil organisms may become exposed simultaneously to different types of feed additives that are assessed separately or the presence of endangered species in the soil habitats of concern may require a precautionary approach (see also EFSA SC, 2016c).

Table 6: *Overview of proposed soil SPU organisms and their SPG dimensions for the 'high margin of safety option'*

SPU-Organism group	Ecological entity	Attribute	Magnitude of tolerable effect	Temporal scale	Spatial scale
Soil microbes	Functional group	Processes	Negligible + extra AF	< days	Site of application
Soil Protozoa	Functional group or population?	Processes or abundance?	Negligible + extra AF	< days	Site of application
Terrestrial plants	Population	Abundance/ biomass	Negligible + extra AF	< days	Site of application
Earthworms	Population	Abundance/ biomass	Negligible + extra AF	< days	Site of application
Soil arthropods	Population	Abundance/ biomass	Negligible + extra AF	< days	Site of application
Other soil invertebrates (*e.g.* enchytraeids, *molluscs, nematodes*)	Population	Abundance/ biomass	Negligible + extra AF	< days	Site of application
Soil vertebrates (*e.g. mole*)	Individual Population	Survival Abundance/ biomass	Negligible + extra AF	< days	Site of application

A.4.1.2. The ecological threshold option for soil organisms

This 'ecological threshold option' (Table 7) assumes that by allowing negligible effects of exposure to a specific (type of) feed additive, the SPU-key organism groups will be sufficiently protected also in case of simultaneous exposure to different types of feed additives. Since the magnitude of tolerable effect is set at negligible for this option, the ecological threshold option seems to be the option that up till now is used by calculating the PEC/PNEC ratio on basis of the most sensitive (standard) test species.

Table 7: *Overview of proposed soil SPU organisms and their SPG dimensions for the 'ecological threshold option'*

SPU-Organism group	Ecological entity	Attribute	Magnitude of tolerable effect	Temporal scale	Spatial scale
Soil microbes	Functional group	Processes	Negligible	< days	Site of application
Soil Protozoa	Functional group or population?	Processes or abundance?	Negligible	< days	Site of application
Terrestrial plants	Population	Abundance/ biomass	Negligible	< days	Site of application
Earthworms	Population	Abundance/ biomass	Negligible	< days	Site of application
Soil arthropods	Population	Abundance/ biomass	Negligible	< days	Site of application
Other invertebrates (e.g. enchytraeids, molluscs, nematodes)	Population	Abundance/ biomass	Negligible	< days	Site of application
Soil vertebrates (e.g. mole)	Individual	Survival	Negligible	< days	Site of application
	Population	Abundance/ biomass			

A.4.1.3. The ecological recovery option for soil organisms

This 'ecological recovery option' (Table 8) allows a local but temporal effect on processes by terrestrial microbes and population structure of terrestrial plants and soil invertebrates, as long as the permissible direct effects do not result in unacceptable indirect effects. Temporal effects on vertebrates are not permissible. Note that when selecting this option the ERA scheme should be protective as well for vulnerable field populations within the SPU-key organism groups. This may not be feasible if organisms at stake are potentially sensitive, have a long and complex life-cycle and a limited dispersal capacity.

Table 8: *Overview of proposed soil SPU organisms and their SPG dimensions for the 'ecological recovery option'*

SPU-Organism group	Ecological entity	Attribute	Magnitude of effect	Temporal scale	Spatial scale
Soil microbes	Functional group	Processes	Small	Months	Site of application
			Medium	Weeks	
			Large	Days	
Soil Protozoa	Functional group or population?	Processes or abundance?	Small	Months	Site of application
			Medium	Weeks	
			Large	Days	
Terrestrial plants	Population	Abundance/ biomass	Small	Months	Site of application
			Medium	Weeks	
			Large	Days	
Earthworms	Population	Abundance/ biomass	Small	Months	Site of application
			Medium	Weeks	

			Large	Days	
Soil arthropods	Population	Abundance/ biomass	Small	Months	Site of application
			Medium	Weeks	
			Large	Days	
Other soil invertebrates (e.g. enchytraeids, molluscs, nematodes)	Population	Abundance/ biomass	Small	Months	Site of application
			Medium	Weeks	
			Large	Days	
Soil vertebrates (e.g. mole)	Individual	Survival	Negligible	< days	Site of application
	Population	Abundance/ biomass			

A.4.2. Dung dwelling fauna

Dung, especially from free-roaming larger mammals but potentially also chicken dung spread on the top-soil (Giner Santonja et al., 2017), makes up a complex and highly dynamic ecosystem. The organisms involved in dung decomposition provide four vital ecosystem services, viz. (1) the removal of dung as a source of pathogens, parasites and pests, (2) the mineralisation of dung and the supply of nutrients to plants, (3) dung fauna as food source for birds and other insectivorous animals, and (4) dung as habitat for endangered dung fauna. In a guideline on the higher tier testing of veterinary medicinal products to dung fauna, the European Medicines Agency (EMA) particularly mentions dung dwelling beetles (among which several endangered species) and flies as taxa to protect (EMA, 2016). In developing SPGs for feed additives these taxa of dung fauna might be taken into consideration as well.

In Table 9 relevant dung fauna SPU organism groups and related standard test species are mentioned. Note that these standard test species at the time of writing this guidance are not a basic data requirement for feed additives.

Table 9: *Overview of dung SPU organisms (in dung of free-roaming grazers like cattle), examples of related standard test species and basic data requirements in the EMA 2016 guideline*

SPU-Organism group	Examples of standard test species /assays	Possible data requirements Feed additives
Dung flies	OECD dung fly larvae test (OECD 228)	No
Dung beetles	OECD dung beetle larvae test (OECD 122)	No

A.4.2.1. The high margin of safety option for dung fauna

The 'high margin of safety option' (see Table 10) assumes that an extra AF should be used when assessing the magnitude of tolerable effects for individual (types of) feed additives, since dung fauna may become exposed simultaneously to different types of feed additives that are assessed separately, or the presence of endangered species in dung pads of concern may require a precautionary approach (see also EFSA SC, 2016c). Since for dung flies no endangered species are mentioned by EMA (2016) their ecological entity to consider is either the functional group or population. Protecting dung flies at the functional group level probably secures the ecosystem services that concern the removal of dung as a source of pathogens, parasites and pests, the mineralisation of dung and the supply of nutrients to plants, and dung fauna as food source for birds and other insectivorous animals. For dung beetles EMA (2016) reports a list of endangered species.

Table 10: *Overview of proposed dung fauna SPU organisms and their SPG dimensions for the 'high margin of safety option'*

SPU-Organism group	Ecological entity	Attribute	Magnitude of tolerable effect	Temporal scale	Spatial scale
Dung flies	Functional group/ Population	Abundance/ biomass	Negligible + extra AF	< days	Dung pads in individual agricultural fields or meadows
Dung beetles	Population	Abundance/ biomass	Negligible + extra AF	< days	Dung pads in individual agricultural fields or meadows

A.4.2.2. The ecological threshold option for dung fauna

This 'ecological threshold option' (Table 11) only differs from the previous option in the 'Magnitude of tolerable effect' dimension and assumes that by allowing negligible effects of exposure to a specific (type of) feed additive, the SPU-key organism groups will be sufficiently protected also in case of simultaneous exposure to different types of feed additives. The effect assessment scheme described in EMA (2016) is more or less in line with this option.

Table 11: *Overview of proposed dung fauna SPU organisms and their SPG dimensions for the 'ecological threshold option'*

SPU-Organism group	Ecological entity	Attribute	Magnitude of tolerable effect	Temporal scale	Spatial scale
Dung flies	Functional group/ Population	Abundance/ biomass	Negligible	< days	Dung pads in individual agricultural fields or meadows
Dung beetles	Population	Abundance/ biomass	Negligible	< days	Dung pads in individual agricultural fields or meadows

A.4.2.3. The ecological recovery option for dung fauna

This 'ecological recovery option' (Table 12) allows a local but temporal effect on the abundance of dung flies and dung beetles, as long as the permissible local direct effects do not result in unacceptable indirect effects at a larger spatial scale (e.g. limited food for insectivorous birds) and the protection of vulnerable populations is guaranteed at a relevant spatial scale of the landscape. Considering the fact that the life-span of dung pads is relatively short (weeks to months) the ecological recovery option for dung fauna needs to be assessed for a larger population of dung pads in the relevant patch of landscape.

Table 11: *Overview of proposed dung fauna SPU organisms and their SPG dimensions for the 'ecological threshold option'*

SPU-Organism group	Ecological entity	Attribute	Magnitude of tolerable effect	Temporal scale	Spatial scale
Dung flies	Functional group/ Population	Abundance/ biomass	Medium	Life-span of dung pad	Dung pads in individual agricultural fields or meadows
			Negligible to small	Life-span of dung pad	Population of dung pads in landscape
Dung beetles	Population	Abundance/	Medium	Life-span of	Individual dung

		biomass		dung pad	pad
			Negligible to small	Life-span of dung pad	Population of dung pads in landscape

A.4.3. Selected SPG option for terrestrial ecosystems (soil organisms and dung fauna)

After the description of the SPG options by the working group, they were presented to the FEEDAP Panel and risk managers of the European Commission. It was argued that feed additives, with the exception of coccidiostats, do not possess endo- or ecto-parasiticidal activities and that therefore in most cases the risks to typical dung fauna need not to be assessed. Furthermore, coccidiostats predominantly will occur in chicken manure that either is worked into the soil or spread on grassland. The members of the working group and the FEEDAP Panel are not aware of typical dung fauna associated with chicken manure applied on the top-soil. Consequently it was decided not to develop specific ERA schemes for dung fauna exposed to feed additives. It was considered that the ERA schemes for feed additives and soil fauna sufficiently cover the possible risks to typical dung fauna.

With respect to the SPG options for soil organisms, risk managers indicated that they require more time to evaluate the proposed SPGs and the procedure to derive them, as well as the possible consequences (cost-benefit analysis) for placing feed additives on the European market. Based on the oral comments received it was decided to select the 'Ecological Threshold Option' as SPG for soil organisms. This option is most in line with the ERA schemes developed for feed additives in the old Technical Guidance. In addition, it is assumed that the environmental risk of typical soil vertebrates (e.g. mole) is suffiently covered with the risk assessment of livestock animals.

A.5. Development of relevant exposure assessment goals (EAGs)

The SPGs developed and selected are mainly defined in eco(toxico)logical terms, and, consequently inform the development of effect assessment schemes (e.g. to derive PNECs) in particular. The overall protection of the environment, however, is determined by the combination of effect and exposure assessment. Just like SPGs are fundamental to inform tiered effect assessment schemes, Exposure Assessment Goals (EAGs) are fundamental to inform tiered exposure assessment schemes. Further explanation on EAGs can e.g. be found at http://pfmodels.org/downloads/EMW7_options_groundwater_protection_goals.pdf.

EAGs can be defined by posing the following questions:

1. What is the ecotoxicologically relevant type of concentration (ERC) to select as 'C' in the PEC? (e.g. the total concentration of the substance in dry soil or the freely dissolved fraction in pore water of soil); this ERC should not be in conflict with that selected for the effect assessment (the ERC for the PNEC and PEC estimates should be the same)
2. What is the temporal dimension of the ERC to select as 'C' in the PEC? (e.g. the maximum peak concentration per year or the highest time-weighted average concentration over an ecologically relevant time-frame, e.g. a 28-d TWA concentration)
3. What is the spatial dimension of the ERC to select as 'C' in the PEC (e.g. the concentration in the upper 5 cm or 20 cm of soil),
4. What are the spatial units for the statistical population of concentrations to consider (e.g. the concentrations in the top-soil of all treated agricultural fields in a specific area, e.g. a region, a Member State, a regulatory zone in the EU)

5. What are the multi-year temporal units for the statistical population of concentrations to consider (e.g. the past 5, 10, 15 climatic years)
6. Which percentile from the statistical spatial-temporal population of concentrations should be selected for the final PEC? (e.g. the overall 90th percentile PEC_{max} or PEC_{twa} in soil)

A.6. Dialogue with risk managers

The SPG options and related EAG options derived for ERA schemes of feed additives (and their major metabolites) are needed for the dialogue with risk managers. The responsibility of risk assessors is (i) to acknowledge existing general protection goals and regulatory data requirements, (ii) to propose possible SPG options and related EAG options, and (iii) to describe the possible environmental consequences of each option. What is a tolerable level of risk, and thus whether a regulated product can be commercialised, is decided by risk managers (EFSA Scientific Committee, 2016a). This means that they may request to adapt the options presented and/or that they select a preferred option. It may also be possible that risk managers prefer that ERA schemes are developed for more than one SPG-EAG option.

As discussed above, risk managers indicated that they require more time to evaluate the proposed SPGs and EAG options, as well as the possible consequences (cost-benefit analysis) for placing feed additives on the European market. Nevertheless, based on the oral input the SPG and EAG options selected for the updated Technical Guidance are in line with the ERA schemes developed for feed additives in the old Technical Guidance. This, however, is made more transparent by the procedure described in this Appendix.

A.7. Developing an ERA scheme for each SPG-EAG combination

Key is that a tiered ERA scheme should be internally consistent. This means that lower tiers require less effort but are more conservative than higher tiers. Higher tiers aim at being more realistic than lower tiers. In each tier all available relevant scientific information is used. All effect assessment tiers within a scheme aim to address the same SPG and all exposure assessment tiers within that scheme aim to address the same related EAG. Lower tiers can be calibrated/validated by higher tiers (see Figure 1).

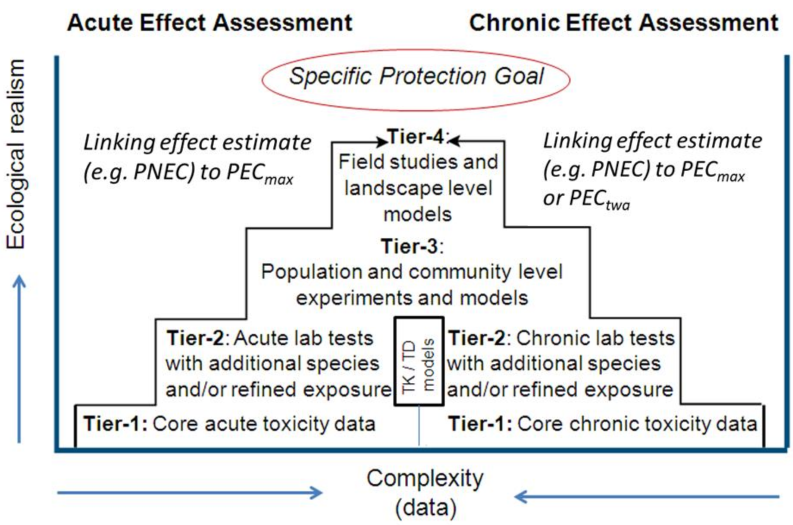

***Figure 1:** Tiers in an effect assessment scheme, showing the refinement of the process through the acquisition of additional data (redrafted after EFSA PPR Panel, 2013)*

For the effect assessment (e.g. PNEC derivation), the tier 1 usually is based on the basic dossier requirements. Since lower tiers should be more conservative than higher tiers, effect estimates (e.g. PNECs) generated at higher tiers should be higher than those at lower tiers. Consequently, higher tier information can be used to validate/calibrate lower tiers. Ideally, the consistency of the different tiers within an ERA scheme should be evaluated for a number of benchmark feed additives.

In a realistic worst-case ERA the linking of exposure (PEC estimates) and effects (e.g. PNEC estimates) is not in conflict by acknowledging the following principles:

a. The effect assessment and exposure assessment is based on the same ERC
b. In both acute and chronic risk assessments the PEC_{max} can be used. Use of the PEC_{max} in chronic ERA can be considered a precautionary worst-case approach
c. In chronic risk assessments under certain conditions the PEC_{twa} may be used
    i. Reciprocity of effects demonstrated/likely
    ii. Toxicity estimates on which the PNEC is based are expressed in terms of (geometric) mean concentrations during the exposure period of the test
    iii. Time-frame of the PEC_{twa} estimate should be shorter that the duration of the exposure periods in the toxicity tests that drive the PNEC
d. Toxicity data that are expressed in terms of initial exposure concentration may be used to derive a PNEC if in the ERA this PNEC is compared with the PEC_{max} and it is likely/plausible that the decline in exposure is not faster in the toxicity tests than that predicted for the field.

A.8. References

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Appendix B – Application of FOCUS models in Ground water

Input parameters PEARL

1. Scenario:

Location: → pick one

Crop Calendar: → *WCEREALS*

Irrigation: → irrigation scenarios are considered for Chateaudun, Piacenza, Sevilla, Thiva; *No irrigation* in the other cases.

Tillage: → No tillage

Repeat interval for application events (a): → 1

2 Simulation Control:

Start date: → 01/01/1901

Stop date: → 31/12/1926

Stop criterion (kg ha-1): → default zero

Repeat hydrology: → no tick

Although the total time is 26 years, the protocol on the reactive tracer will be for only 20 years.

3 Output Control:

Summary report: → pick FOCUS report

No additional changes.

4 Swap Hydrological Method:

Option Hydrology: → Run SWAP and then PEARL only

No additional changes.

5 Substance:

General

Molar mass (g/mol): → enter value

Saturated vapour pressure (Pa): → enter value

Molar enthalpy of vapourisation (kJ/mol): → 95 (default pesticides)

Solubility in water (mg/l): → enter value

Molar enthalpy of dissolution (kJ/mol): → 27 (default pesticides)

Freundlich sorption

K_{OM}: → enter value ($K_{OM} = K_{OC} / 1.724$)

No additional changes.

Transformation

Half-life (d): → enter value

No additional changes.

Diffusion

No changes, use default settings from pesticides.

Crop

Wash-off factor (m^{-1}): → $\geq 10^{-6}$, even if there is no wash-off.

Coefficient for uptake by plant: → no uptake

<u>6 Application</u>

Advice should be given, which application form is most appropriate for feed additives. Since for feed additives, either arable land or grassland without harvest are considered, *absolute application* seems more appropriate than relative application.

As the input in FOCUS is expressed in kg/ha, the PEC soil has to be converted using the following equation (see also formula in Section 1.1.1):

$$ApplRate = \frac{PEC_{soil} \cdot DEPTH_{field} \cdot RHO_{soil}}{100}$$

Where:

Symbol	Parameter	Default Value	Unit
Input			
PEC_{soil}	Concentration of the additive (parent compound) in soil (dry weight)		mg/kg $soil_{dw}$
RHO_{soil}	Bulk density of (dry) soil	1,500	kg/m^3
$DEPTH_{field}$	Mixing depth with soil	0.05	m
	Conversion factor	100	mg/kg × ha/m^2
Output			
ApplRate	Application rate		kg/ha

Absolute applications

Application type: → either *incorporation* or *application to the soil surface*

Date: → enter date of application (pre-emergence)

Dosage (kg/ha): → enter value

Depth (m): → default 5 cm for $PEC_{soil;tot}$ 20 cm for $PEC_{soil;pw}$ (realistic worst case)

<u>7. Deposition</u>

No deposition

Appendix C – Application of FOCUS models in surface water

SWASH

1 Actions/ Create view and edit substances

General:

Enter information on chemical properties (molar mass, vapour pressure, solubility in water, metabolism).

For molar enthalpy of vaporisation and dissolution and diffusion coefficients in water and air the default values from pesticides may be used.

Maybe a short comment regarding the applicability of the default values especially to macromolecules should be inserted, since these properties are generally assumed to be substance specific.

Sorption:

Enter either K_{OM} or K_{OC}, the other value will be calculated internally.

Enter Freundlich exponent. (The corresponding Freundlich exponent for soil or sediment is internally calculated from the given K_{OM} or K_{OC} value and the fraction of organic matter in the soil of the chosen scenario.)

Reference concentration in the liquid phase [g/m³]: This refers to the concentration at which the sorption parameters were determined. If it was at 1 g/m³, then the default value of 1 is correct. In case the concentration was significantly different from 1 g/m³, the appropriate value should be inserted. This is then used for internal correction of the Freundlich parameters.

Uptake and wash-off:

Do not assume any plant/ root uptake or wash off. Hence, set all parameters zero.

Transformation:

Enter DT_{50} in water, soil and sediment and the respective temperatures.

If you assume no transformation in the crop (or no data are available), set a large DT_{50} in crop (e.g. 10^3).

Effect of temperature: Use default value from pesticides if no data are available.

Specifications on transformation in soil: Use default values from pesticides for the dependence of transformation on soil moisture/ water content.

2. Focus wizard

Use Wcereals for crops selection. Although more realistic, a pure grassland scenario is not available. Root uptake zero has to be set to zero (in the window "uptake and wash off").

3 User defined wizard

Selected crop according to the chosen crop above.

Accept selected water body types.

Accept appropriate scenarios.

4 View projects and define applications

View and edit application: Enter number of applications, as well as the application mode (granular application is the closest scenario to manure spreading). For run-off scenarios the depth of incorporation is also required.

Appendix D – Quantitative structure-activity relationships calculations

Data requirements and quantitative structure-activity relationships calculations in Phase I

In the absence of experimental data the physical chemical and fate properties needed as screening information in phase I can be estimated using non-testing approaches, such as QSARs. In the ideal situation, (Q)SAR results can be used on their own provided they are relevant, reliable and adequate for the purpose, and if they are documented in an appropriate manner. These aspects are further discussed in the OECD guidance document on the validation of QSAR models, the OECD guidance on grouping of chemicals and the ECHA guidance on QSARs and grouping of chemicals. The careful use of expert judgement to define the boundaries of a chemical category is crucial to the reliable application of QSAR models or other methods to estimate values for untested chemicals. For instance for ionisable active substances, the proper QSAR should be used when the active substance can be ionised between pH 3 and 9 (common soil pH values).

One of the QSAR tools that can be used is the EPI (Estimation Programs Interface) Suite™ of the US Environmental Protection Agency (USEPA), which uses as input a simplified molecular-input line-entry system (SMILES) notation to run different programs to estimate the physical-chemical and fate properties. In the EPI Suite™, the organic carbon partitioning coefficient (K_{oc}) can be estimated using the Molecular Connectivity Index (MCI) method or the octanol-water partition coefficient (log K_{ow}) methodology. The MCI method is overall somewhat more accurate than the log K_{ow} method. As a first worst-case estimate for the leaching of compounds to groundwater the lowest K_{oc} should be selected.

The Biowin models can be used to screen whether a chemical potentially meets the P criterion in the PBT assessment, as outlined in appendix E. To determine whether a chemical could accumulate over multiple year application, a first rough estimate of the aerobic half-life (DT_{50} soil) at room temperature can be made using the rating number provided by BioWin3 (Ultimate Survey Model) in the formula developed by Arnot et al. (2005):

$$DT50 = 10^{(-1.07r+4.12)}$$

Although this DT_{50} soil is considered a rough estimate of the ultimate degradation of an active substance to minerals and carbon dioxide, it can be used to calculate a refined PEC for persistent active substances. In case that r<2.5, corresponding with a half-life of more than 28 days at room temperature, further experimental data on the biodegradability of the compound is needed and the assessment should go to phase II. For more rapidly degradable compounds the half-life does not play an important role in the calculation of the initial PEC_{pw} in Phase I since no degradation is assumed.

QSAR calculations to estimate ecotoxicity in Phase II

Generally, experimental data from GLP accredited toxicity studies should be available for Phase II. In some specific circumstances, the FEEDAP Panel might allow the use of QSAR derived data.

The ecological structure activity relationship (ECOSAR) program within the EPI Suite™ developed by the US EPA is one of the tools that can be used to estimate the half-maximal effective concentration (EC_{50}) or lethal concentration (LC_{50}) for earthworms, fish, green algae and daphnids. Like for the other QSARs, it should be checked whether the QSAR model selected by ECOSAR is appropriate. The default QSAR for "neutral organic" active substances should only be used for active substances where minimal toxicity can be expected based on the chemical structure.

To cover the uncertainty on the QSAR prediction the PNEC for the aquatic compartment ($PNEC_{sw}$) can be derived by selecting the lowest predicted toxicity value (obtained from the QSAR dataset of short term studies for daphnids, green algae and fish) for aquatic organisms and by applying an extra AF of 10 to the AF of 1,000 that is applied to experimental data. This additional AF can be lowered when additional information compensates for uncertainties resulting from the uncertainty on the (Q)SAR.

In the absence of experimental terrestrial toxicity data, the equilibrium partitioning method can be applied to calculate the $PNEC_{soil}$ from the $PNEC_{sw}$. The method assumes that toxicity in the soil, expressed as the concentration in pore water, is the same as toxicity measured in water-only exposure. Consequently, soil organisms show similar species sensitivity distributions (EC_{50} or LC_{50} expressed in µg/L pore water) than aquatic organisms (EC_{50} or LC_{50} expressed in µg/L surface water). When a $PNEC_{sw}$ is estimated from the aquatic toxicity tests, this value can be used to calculate a

$PNEC_{soil}$.

$$PNEC_{soil} = \frac{K_{soil-water}}{RHO_{soil}} \times PNEC_{surfacewater} \times 1000$$

The RHO dry soil of 1,500 kg/m^3 can be used. In addition, the $PNEC_{soil;total}$ can be derived from a QSAR from earthworms which is also available in ECOSAR for a number of active substances. The LC_{50} for earthworms is divided by 1,000 and should only be used when it is lower than the $PNEC_{soil;total}$ derived from the equilibrium partitioning method.

An environmental risk assessment based on QSAR data can only be used for screening purposes to decide for which compounds more data should become available.

Example of risk assessment using QSARs

The following example aims to illustrate how the output of EPI Suite™ for myrcene is used. It is assumed that the concentration in feed is 5 mg a.i./kg. From the CAS number, the program derives the SMILE notation based on which the physico-chemical properties are estimated (see table D1). The EPI Suite™ database had an experimental solubility of 4 mg/l available which is preferred over the calculated solubility. Note that EPI Suite™ estimated a log K_{oc} of 3.031 using the MCI method and a log K_{oc} of 3.758 using the K_{ow} method. The lowest K_{oc} calculated via the MCI method and the K_{ow} method is selected to calculate the concentration in pore water and surface water. The BioWin3 model gives an outcome of 2.8981, based on which the estimated DT_{50} in soil is 10 days at room temperature which gives a DT_{50} of 22 days at 12 °C, indicating that the active substance does not accumulate over the years and that the initial PEC can be used as a reasonable worst case. The PECs are calculated as described above in this guideline. The calculations are performed for pigs for fattening (see Appendix F) because these give the highest PEC_{soil} at a given feed dose, compared to the other animal categories.

Table D1. The physicochemical properties predicted by EPISUITE 4.1 for myrcene

EU Register name	CAS No.	Predicted by EPIWEB 4.1				
		DT_{50}(1) (days)	Molecular weight (g/mol)	Vapour pressure (Pa)	Solubility (mg/L)	K_{oc}(2) (L/kg)
Myrcene	123-35-3	10	136.24	320	4	1074

EU: European Union; CAS No: Chemical Abstracts Service.
(1): DT_{50}, half-life of the additive at room temperature (EPI 4.1.BioWin3).
(2): K_{oc}, organic carbon sorption constant (EPI 4.1.KocWin2.0).

When the toxicity data is based on QSARs the PNEC for the aquatic compartment ($PNEC_{sw}$) is derived from the lowest toxicity value for freshwater organisms by applying a AF of 10,000. To derive the $PNEC_{soil}$ there are two options: The LC_{50} for earthworms divided by a AF of 10,000 or the equilibrium partitioning method using the $PNEC_{sw}$. The $PNEC_{soil}$ from the equilibrium partitioning method is much lower than the $PNEC_{soil}$ from the earthworm QSAR. Generally the approach should be over conservative to invite applicants to provide real data.

Table D2. Phase II environmental risk assessment of myrcene in soil and aquatic compartments (Exposure and effect data were modelled using EPISUITE 4.1 and ECOSAR 2.0)

Soil	LC_{50}(1) Earthworm (mg/kg)	$PNEC_{soil}$ (µg/kg)	PEC_{soil} (µg/kg)	PEC/PNEC
myrcene	119	11.9	101	8

Aquatic	LC_{50} Fish (mg/L)	LC_{50} Daphnids (mg/L)	EC_{50}(2) Algae (mg/L)	PNEC(3) aquatic (µg/L)	PEC_{sw}(4) (µg/L)	PEC/PNEC
myrcene	0.292	0.216	0.483	0.0216	0.4	18

Soil using $PNEC_{aquatic}$	**$PNEC_{aquatic}$**	**$K_{soil\ water}$ (L/kg)**		**$PNEC_{soil,\ EP}$ (µg/kg)**	**PEC_{soil} (µg/kg)**	**PEC/PNEC**
	0.0216	21		0.45	101	223

2231 EU: European Union.
2232 (1): LC_{50}, the concentration of a test substance which results in a 50% mortality of the test species.
2233 (2): EC_{50}, the concentration of a test substance which results in 50% of the test animals being adversely affected (i.e. both
2234 mortality and sublethal effects).
2235 (3): Experimental data selected in preference to modelled data for derivation of the PNEC
2236 (4): PEC_{sw}: Predicted environmental concentration in surface water.

This example shows that a concentration of 5 mg a.i./ kg feed will pose a risk for both the aquatic and terrestrial environment. Table D3 shows the concentrations in feed that result in PECs not exceeding the PNEC for the terrestrial and aquatic environment and the groundwater trigger of 0.1 µg/L. Based on this first screening a dose of 0.02 mg/kg could be considered safe for all compartments, which can be refined when experimental data becomes available.

Table D3. Doses of example substance safe for different compartments

Dose mg/kg feed	**PEC_{soil} (µg/kg)**	**$PEC_{pore\ water}$ (µg/L)**	**PEC_{sw} (µg/L)**	**Safe for Compartment**
0.02	0.45	0.005	0.002	terrestrial EP
0.29	6	0.07	0.0216	Aquatic
0.59	11.9	0.13	0.045	Terrestrial

EP: equilibrium partitioning

Appendix E – Screening information for Persistence, Bioaccumulation and Toxicity

1. Screening information for Persistence, Bioaccumulation, and Toxicity

Table E1. Screening criteria according to ECHA (2017e)Part C: PBT/vPvB assessment.Section C.4.1.

Type of screening information	Screening criterion	Conclusion
Persistence		
Biowin 2 (non-linear model prediction) and Biowin 3 (ultimate biodegradation time)	Does not biodegrade fast (probability <0.5)[(a)] and ultimate biodegradation timeframe prediction: ≥ months (value < 2.25 (to 2.75)[b])	Potentially P or vP
or	**or**	
Biowin 6 (MITI non-linear model prediction) and Biowin 3 (ultimate biodegradation time)	Does not biodegrade fast (probability <0.5)[(a)] and ultimate biodegradation timeframe prediction: ≥ months (value <2.25 (to 2.75)[(b)])	Potentially P or vP
or	**or**	
other models [(a)]	Model specific values	Potentially P or vP
Ready biodegradability test (including modifications allowed in the respective TGs)	≥70% biodegradation measured as DOC removal (OECD TGs 301A, 301E and 306) or ≥60% biodegradation measured as ThCo2 (OECD TG 301B) or ThOD (OECD TGs 301C, 301D, 301F, 306 and 310) [(c)]	Not P and not vP
	<70% biodegradation measured as DOC removal (OECD TGs 301A, 301E and 306) or <60% biodegradation measured as ThCo2 (OECD TG 301 B) or ThOD (OECD TGs 301C, 301D, 301F,306 and 310)	Potentially P or vP
Enhanced screening tests [(d)]	biodegradable	Not P and not vP
	not biodegradable [(d)]	Potentially P or vP

Specified tests on inherent biodegradability:		
- Zahn-Wellens (OECD TG 302B)	≥70 % mineralisation (DOC removal) within 7 d; log phase no longer than 3d; removal before degradation occurs below 15%; no pre-adapted inoculum	Not P and not vP
	Any other result (e)	Potentially P or vP
- MITI II test (OECD TG 302C)	≥70% mineralisation (O2 uptake) within 14 days; log phase no longer than 3d; no pre-adapted inoculum	Not P and not vP
	Any other result (e)	Potentially P or vP
Bioaccumulation		
Octanol-water partitioning coefficient (experimentally determined or estimated by QSAR)	Log Kow ≤ 4.5	not B and not vB (f) (in aquatic organisms)
	Log Kow > 4.5	Potentially B or vB (in aquatic organisms)
Combination of the Octanol water partitioning coefficient with the octanol air partitioning coefficient (both experimentally determined or estimated by QSAR)	Log Kow > 2 and Log Koa > 5	Potentially B (in air-breathing organisms)
Toxicity		
Short-term aquatic toxicity (algae, daphnia, fish)	EC_{50} or LC_{50} < 0.01 mg/L (g)	T criterion considered to be definitely fulfilled
Short-term aquatic toxicity (algae, daphnia, fish)	EC_{50} or LC_{50} < 0.1 mg/L (g)	Potentially T

(a) The probability is low that it biodegrades fast (see Section R.7.9.4.1 in Chapter R.7b of the Guidance on IR&CSA). Other models are described in Section R.7.9.3.1 in Chapter R.7b of the Guidance on IR&CSA and in this section below.
(b) For substances fulfilling this but BIOWIN 3 indicates a value between 2.25 and 2.75 more degradation relevant information is generally warranted.
(c) These pass levels have to be reached within the 28-day period of the test. The conclusions on the P or vP properties can be based on these pass levels only (not necessarily achieved within the 10-d window) for monoconstituent substances. For multi-constituents substances and UVCBs these data have to be used with care as detailed in Section R.11.4.2.2 of Chapter R.11 of the Guidance on IR&CSA.
(d) See Sections R.7.9.4 and R.7.9.5 in Chapter R.7b of the Guidance on IR&CSA. Expert judgement and/or use of Weight of Evidence also employing other information may be required to reach a conclusion (i.e. concerning « biodegradable/not biodegradable »).
(e) See section below for concluding ultimately on persistence in particular cases (in particular "Tests on inherent biodegradation").
(f) Care must be taken and a case-by-case assessment made if a substance is known to bioaccumulate by a mechanism other than passive diffusion driven by hydrophobicity. E.g. specific binding to proteins instead of lipids might result in an erroneously low bioaccumulation potential if it is estimated from Log Kow.
Care must also be taken for substances classified as polar non-volatiles (with low Log Kow and high Log Koa). This group of substances has a low bioaccumulation potential in aquatic organisms but a high bioaccumulation potential in air-breathing organisms (unless they are rapidly metabolised).
(g) These threshold values only apply for the aquatic compartment.

2. PBT and vPvB criteria according to Annex XIII to REACH Property

Table E2. PBT and vPvB criteria according to ECHA (2017e) Guidance on information requirements and chemical safety assessment, Part C: PBT/vPvB assessment:

Property	PBT-criteria	vPvB-criteria
Persistence	A substance fulfils the persistence criterion (P) in **any** of the following situations: • $T_{1/2}$ > 60 days in marine water; • $T_{1/2}$ > 40 days in fresh- or estuarine water; • $T_{1/2}$ > 180 days in marine sediment; • $T_{1/2}$ > 120 days in fresh- or estuarine sediment; • $T_{1/2}$ > 120 days in soil.	A substance fulfils the "very persistent" criterion (vP) in **any** of the following situations: • $T_{1/2}$ > 60 days in marine, fresh- or estuarine water; • $T_{1/2}$ > 180 days in marine, fresh- or estuarine sediment; • $T_{1/2}$ > 180 days in soil.
Bioaccumulation	A substance fulfils the bioaccumulation criterion (B) when: BCF > 2000	A substance fulfils the "very bioaccumulative" criterion (vB) when: BCF > 5000
Toxicity	A substance fulfils the toxicity criterion (T) in **any** of the following situations: • NOEC or EC_{10} < 0.01 mg/L for marine or freshwater organisms; • substance is classified as carcinogenic (category 1A or 1B), germ cell mutagenic (category 1A or 1B), or toxic for reproduction (category 1A, 1B or 2); • there is other evidence of chronic toxicity, as identified by the classifications: STOT (repeated exposure), category 1 (oral, dermal, inhalation of gases/vapours, inhalation of dust/mist/fume) or category 2 (oral, dermal, inhalation of gases/vapours, inhalation of dust/mist/fume) according to the CLP Regulation.	-

Appendix F – Concentration of a feed additive (mg/kg feed) that would correspond to a PEC below the trigger value for the different species

Table F1. Feed intake and nitrogen excretion cause different manure concentrations for the animal categories

Animal category[1]	Feed intake (kg/ animal place and year)[1]	N excretion (kg/ animal place and year)[2]	Concentration in mg/kg feed resulting in a PEC of 10µg/kg soil	PEC manure in mg/kg Nitrogen from 1 mg/kg feed
Pig for fattening	800	9	0.5	89
Cattle for fattening	4050	54	0.6	75
Piglet	296	4	0.6	74
Turkey for fattening	70	1	0.6	70
Chicken for fattening	22	0.33	0.7	67
Veal calf	730	11	0.7	66
Horse	3650	58	0.7	63
Meat sheep	607	10	0.7	61
Rabbit for fattening	30	0.5	0.7	60
Dairy sheep	580	10	0.8	58
Horse for fattening	2385	43	0.8	55
Lamb for fattening	273	5	0.8	55
Sheep for fattening	267	5	0.8	53
Dairy cow	6584	125	0.8	53
Laying hen	42	0.8	0.8	53
Sow with piglets	1140	23	0.9	50
Dairy goat	714	16.4	1.0	44

1) For the characteristics of these animal categories refer to Table 1 of the guidance document.

The ratio between the feed intake and the nitrogen excretion determines the PEC manure. A dose of 1 mg feed additive/kg feed results in different manure concentrations in the different species/categories expressed in mg/kg nitrogen in manure. Note that the animal categories in table F1 are ordered for a decreasing PEC manure resulting from 1 mg/feed additive/kg feed. This indicates that a dose that causes no environmental concern for pig for fattening will not cause an environmental concern for the other animal categories. This is based on the assumption in Phase 1 of the risk assessment that there is no metabolism of the feed additive.

The feed concentrations in fourth column of table F1 all result in a PEC manure of 44 mg/kg nitrogen and therefore in a PEC soil of 10 µg/kg soil.

Appendix G – Rationale for the proposal to increase the nitrogen load to agricultural land form manure application from 170 to 250 kg N/ha per year

The FEEDAP panel re-considered the nitrogen load load to agricultural land from manure application which was set as a standard value for the calculation of the PEC_{soil} according to the Technical Guidance for assessing the safety of feed additives for the environment from 2008 (EFSA, 2008b). In the guidance it was stated that: "The amount of manure/slurry containing the feed additives allowed to be spread on land depends on the nitrogen content of the manure and the nitrogen load standard". The standard load of 170 kg N/ha was set according to the Nitrate Directive (European Commission, 1991) to the maximum allowed annual amount of nitrogen originating from animal manure on a farm within nitrate vulnerable zones (NVZ) .

In order to prevent underestimation of the exposure of feed additives to the primary receiving terrestrial compartment, the FEEDAP panel notes that predicted environmental concentrations in soil would be more realistic if instead of the nitrogen standard load (170 kg N/ha per year) a value of about 250 kg N/ha per year is used due to following reasons:

- In the accordance with the Nitrate Directive (European Commission, 1991), NVZ are designated in order to protect the groundwater against the pollution with nitrates. Member states designated different portions of their territory as NVZ. According to the data from Eurostat (EUROSTAT, 2009) some Member states such as Denmark, Germany, Austria, Ireland, Latvia, Luxemburg, Malta, Netherland, Austria, Slovenia and Finland designated all their territory as NVZ. On the other hand, in several member states NVZ covers around or less than 10 % of the total state territory (Poland 1.5%). In average the NVZ covers less than 41% of total area of the territory of the EU Member states. In addition, some member states applied for derogation (Netherlands, Denmark, Germany UK and parts of Belgium and Italy) allowing to use 230-250kg N/ha per year. Consequently, it is difficult to justify the value of 170kgN/ha per year as a standard load value for all of arable land and grassland in EU Member states as it applies to less than 30% of total area of the territory of the EU Member states.

- To ensure the protection of the water bodies, the Nitrate directive set the maximum nitrogen load value of 170 kg N/ha per year for each farm or livestock unit per year. However, within the farm/livestock unit, the amount of applied manure on a field with a particular crop can be substantially higher. Namely, the value of 170 kg N/ha per year is an average load that applies to the entire farm, while some crops need for their growth and development substantially more nitrogen. According to the good agricultural practice for the use of manure on the NVZ, it is possible to spread more than 170 kg N/ha per year, however, the all-over sum for nitrogen on the farm should not exceed that nitrogen standard.

  For example, the most important fodder plants in EU, the maize for grains and the green maize, require for normal development and growth from 230 to 250 kg N/ha per year, while the application to the grassland can reach up to 300 kg N/ha per year (Kristensen, 2015)(Bundesministerium für Land- und Forstwirtschaft Umwelt und Wasserwirtschaft (Oesterreich), 2012)(Sušin, Jože, & Helena, 2016). The terrestrial compartment is exposed to a dose of a feed additive that is applied to the field with the certain crop, not to an average dose for the whole farm. Therefore, soil microbial communities, soil fauna and plants on the field with maize or grassland are exposed to the manure corresponding to the nitrogen load of 230 to 300kgN/kg per year.

- The farm/ livestock unit is not an environmental entity, while the size of a farm in EU Member states varies substantially. The species and communities on the fields with nitrogen high demand crops can be exposed to the higher annual load of manure than average farm load of 170kgN/ ha per year. The value of 250kgN/ha per year for N-load on the field with corn was therefore considered as realistic worth case scenario for the potential exposure to feed additives applied with the manure.

- The FOCUS emission scenarios used to refine the $PEC_{GW/SW}$ values are refereeing to the field with the crop, not to the whole farm/livestock unit. Consequently, the nitrogen load to the field with crop would be a more scientifically sound way of calculation of exposure of terrestrial compartment than the use of an average value of 170kgN/ha per year that applies to farm/livestock unit.

Nitrogen vulnerable zones in 27 Member States (Eurostat, 2009)

	Total Area (1)	Area NVZ (3)	
	(1000 km²)	(1000 km²)	%
EU-27	4 325	1 771	40.9
BE	31	21	67.8
BG	111	59	53.1
CZ	79	31	39.8
DK (2)	43	43	100.0
DE (2)	357	357	100.0
EE	45	3	7.5
IE (2)	70	70	100.0
EL	132	32	24.2
ES	505	64	12.6
FR	549	250	45.6
IT	301	38	12.6
CY (4)	9	1	6.8
LV	65	8	12.7
LT (2)	65	65	100.0
LU (2)	3	3	100.0
HU	93	43	45.8
MT (2)	0	0	100.0
NL (2)	37	37	100.0
AT (2)	84	84	100.0
PL	313	5	1.5
PT	92	3	3.7
RO	238	16	6.7
SI (2)	20	20	100.0
SK	49	16	33.5
FI (2)	338	338	100.0
SE	450	68	15.0
UK	244	94	38.7

(1) Eurostat, LUCAS, 2009

(2) Implementation of an Action Programme on the whole territory in accordance with art 3 (5) of the Nitrates Directive; this does not necessarily mean that the whole territory is nitrate vulnerable according to Article 3 (2) of the Nitrates Directive;

(3) Based on information made available to the Commission in digital form. The estimate of designated area does not include some designations communicated in paper form only;

(4) According to Protocol 10 of Accession the application of the acquis communautaire is suspended in the areas of the Republic of Cyprus not under the effective control of the Government of the Republic

Special values:

0 Less than half the final digit shown and greater than real zero

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Appendix H – Calculations and assumptions made to update the values of feed intake and nitrogen excretion of different animal species/categories

The default values for the calculation of PEC_{manure} and PEC_{soil} of Table 1 were reviewed by:

- characterising the animal species/category in terms of body weight and production cycle;
- calculating the corresponding feed intake, protein input via feed, the fraction of nitrogen ingested (nitrogen = protein x 0.16) that is retained by the animal, and the nitrogen excreted.

The calculations are based in a series of assumptions that are described for the different animal species/categories. It is recognised that is difficult to set a single default value for FI and N excretion given the variety of diets, animal breads, production systems… The aim was to set a single value for FI and N excretion that covers a realistic worst-case scenario.

The following acronyms were used:

- BW: body weight
- FI: feed intake
- Run: production cycle
- CP: crude protein
- N : nitrogen

1) Piglet

BW (range)	BW gain (kg)	FI/run (kg)	runs/year	% CP	N in feed	N ingested (kg/year)	N retained/ ingested	N excreted (Kg/year)	FI/animal place and year
7-30 kg	23	40	7.4	20	3.2	9.5	0.6	3.8	296

Assumptions:

- N retained from N ingested is 60% (Ju et al., 2008)

2) Pig for fattening

BW (range)	BW gain (kg)	FI/run (kg)	runs/year	% CP	N in feed (%)	N ingested (kg/year)	N retained/ ingested	N excreted (Kg/year)	FI/animal place and year
30-115	85	250	3.2	17	2.7	21.8	0.58	9.1	800

Assumptions:

- N retained from N ingested is 58% (Lee et al, 2016)

3) Chicken for fattening

BW (range)	BW gain (kg)	FI/run (kg)	runs/year	% CP	N in feed (%)	N ingested (kg/year)	N retained/ ingested	N excreted (Kg/year)	FI/animal place and year
0-2.2	2.2	3.4	6.5	20	3.2	0.7	0.6	0.3	22

Assumptions:

- Cobb 500 breed, males and females
- 2.2 Kg weight gain during a production cycle that lasts for 35 days
- Cleaning period between production cycles stablished at 21 days
- N retained from ingested is 60% (Moss et al., 2017)

4) Turkey for fattening

BW (range)	BW gain (kg)	FI/run (kg)	runs/year	% CP	N in feed (%)	N ingested (kg/year)	N retained/ ingested	N excreted (Kg/year)	FI/animal place and year
0-13	13	26.7	2.6	20	3.2	2.2	0.52	1.1	70

Assumptions:

- BUT 6 breed, males and females
- 13 kg average weight (10 kg females and 16 kg males) in a production cycle of 17 weeks
- Cleaning period between production cycles stablished at 21 days
- Feed to gain in males is 1.98 and in females 2.17 kg feed/kg bw.
- N retained from ingested is 60% (Jankowski et al., 2013)

5) Rabbit for fattening

BW (range)	BW gain (kg)	FI/run (kg)	runs/year	% CP	N in feed (%)	N ingested (kg/year)	N retained/ ingested	N excreted (Kg/year)	FI/animal place and year
0.5-2.4	1.9	6.3	4.7	16.5	2.6	0.8	0.39	0.48	30

Assumptions:

- Production cycle of 72 days (from day 28 –weaning- to day 90 –slaughter) FAO
- Cleaning period established at 5 days
- Body weight gain of 1.9 kg
- Feed to gain ratio 3.3 kg feed/kg body weight (Guidenne et al., 2017)
- N retained from ingested is 39% (Birolo et al 2016)

6) Cattle for fattening

BW (range)	BW gain (kg)	FI/run (kg)	runs/year	% CP	N in feed (%)	N ingested (kg/year)	N retained/ ingested	N excreted (Kg/year)	FI/animal place and year
250-630	380	3375	1.2	15	2.4	97.2	0.45	53.5	4050

Assumptions:

- Production cycle of 10 months (from 250 kg to 630 Kg body weight)
- Feed to gain ration of 8.9
- N retained from ingested is 45% (Van Dung et al, 2013)

7) Veal calf

BW (range)	BW gain (kg)	Feed type	FI/run (kg)	runs/year	% CP	N in feed (%)	N ingested (kg/year)	N retained/ ingested	N excreted (Kg/year)	FI/animal place and year
250-630	205	milk replacer	345	1.5	20	3.2	16.6	0.41	9.8	518
		maize grain	99	1.5	9	1.4	2.1	0.41	1.3	149
		maize silage	43	1.5	3	0.5	0.3	0.41	0.2	64
								TOTAL	11.2	731

Assumptions:

- Italian production system (Dell'Orto et al, 2009), based on a study using 6700 veal calves Holstein Friesland males

- Production cycle of 8 months
- Main diet consisting in milk replacer containing 20% crude protein
- Solid diet representing 142 kg/production cycle, consisting in maize grain (70%) and maize silage (30%)
- N retained from ingested is 41% (Gorrill and Nicholson, 1969)

8) Lamb for fattening

BW (range)	BW gain (kg)	Production Phase	FI/run (kg)	runs/year	% CP	N in feed (%)	N ingested (kg/year)	N retained/ ingested	N excreted (Kg/year)	FI/animal place and year
4.0 - 32	28	4 - 11.5 kg (in milk)	35	1.5	25	4.0	2.1	0.4	1.3	53
		11.5 -32 kg (solid feec	123	1.5	14	2.2	4.1	0.3	2.9	185
								TOTAL	4.2	237

Assumptions:

- Production cycle of 4.5 months divided in two phases: milk feeding (1 month) and solid feed feeding (last 3.5 months)
- Milk feeding: milk replacer (35 kg/lamb) containing 25 % CP (Merck veterinary manual). Body weight gain is 0.25 kg/day in the first month (7.5 kg) (EBLEX, Agriculture and Horticulture development board -UK, 2014). Nitrogen retained from ingested is 40%.
- Solid feeding: concentrate containing 14% crude protein, considering a feed to gain ratio of 6 kg concentrate/kg bw gain. Nitrogen retained from ingested is 30% (Tripathi et al, 2006) to 25% (Neville et al., 2010)

9) Sheep for fattening

BW (range)	BW gain (kg)	FI/run (kg)	runs/year	% CP	N in feed (%)	N ingested (kg/year)	N retained/ ingested	N excreted (Kg/year)	FI/animal place and year
15-55	40	178	1.5	16	2.6	6.8	0.3	4.8	267

Assumptions:

- Mean daily feed intake of 1.2 kg
- Daily weight gain of 0.27 kg
- N retained from ingested is 30%

10) Sow

Animal	BW (kg)	Phase	FI/year (kg)	% CP	N in feed (%)	N ingested (kg/year)	N excreted (Kg /animal place and year)
Sow	200	lactating	336	16.5	2.6	8.9	
		non lactating	804.06	14	2.2	18.0	
		total	1140.06			26.9	22.8
product	BW (range)	BW gain	piglets/year	Kg piglet/year	Prot/kg bw	kg N in piglets/year	
piglet	1.5 to 7	5.5	28	154	0.1675	4.1	
Kg creep feed/piglet	kg creep feed	%CP	N in feed (%)	N ingested (kg/year)	N excreted (Kg N/year)	N retained/ ingested	
1	28	23	3.68	1.0304	0.41	0.6	

Assumptions:

- French production system (IFIP, Institut du porc, 2015)
- Lactation lasting 28 days/production cycle
- 2.4 production cycles/year, resulting in 67 lactation days, 278 pregnancy days and 19 days weaning to conception period. 28 piglets/year
- Daily feed intake of non-lactating period is 2.7 kg.

11) Dairy cow

	Cow feed consumption	Phase	days	Time conversion factor	converted days	kg DM/day	FI/phase (kg)
		Peak lactation	120	0.92	111	22	2439
		Late lactation	210	0.92	194	18.5	3590
		Non lactating	60	0.92	55	10	554
		Total cycle (d)	390			Kg feed (DM)	6584
Animal	BW (kg)	Phase	FI/year (kg DM)	% CP	N in feed (%)	N ingested (kg/year)	N excreted (Kg N/year)
cow	650	Peak lactation	2439	17	2.7	66.4	
		Late lactation	3590	15.5	2.5	89.0	
		Non lactating	554	13	2.1	11.5	
		TOTAL feed	6584			166.9	124.7
product	Kg milk/year	% Prot milk	kg N in milk/year				
milk	8000	3.2	40.96				
product	Veal/year	BW (at birth)	Prot/kg veal	kg N in veal/year			
veal calf	0.92	45	0.19	1.26			

Assumptions:

- Production cycle of 13 months

- 0.92 Veals produced per year (one every 13 m)
- Pick lactation phase of 120 days with a feed consumption of 22 kg DM/day
- Late lactation phase of 210 days with a feed consumption of 18.5 kg DM/day
- Non lactating (dry phase) of 60 days with a feed consumption of 14 kg DM/day
- Veal body weight at birth is 45 kg and contains 19% protein

12) Meat sheep (sheep producing lambs for meat)

Production phase	% bw to calculate FI	Kg feed DM/day	days	kg feed DM	% CP	kg protein	kg nitrogen ingested/year	Kg N excreted/year
Ewe maintenance	2	1.2	50	60	9	5.4		
Flushing	2.5	1.5	57	85.5	9.4	8.0		
15 w gestation	2	1.2	105	126	9.4	11.8		
16 - 19 w gestation	2.4	1.44	33	47.52	11	5.2		
Lactation	4	2.4	120	288	13	37.4		
			365	607.0		67.9	10.9	9.8
Product	kg/year	Kg N from product/year						
wool	2	0.17						
milk	70	0.60						
lamb	8	0.26						
		1.03						

Assumptions,

- Suffolk ewe of 60 kg body weight
- Yearly milk production of 70 kg
- Milk containing 5.4% protein
- 2 Lambs per year
- Body weight of lamb at birth is 4 kg
- Yearly wool production is 2 kg, containing 33% keratin and 25% N in keratin.

13) Dairy sheep (milk/cheese production)

Stage of production	months	Kg alfalfa/ day	kg alfalfa/ run	kg protein alfalfa	Kg maize/ day	kg maize/ run	kg protein maize	Kg N intake/year	Kg N excreted/year
Early lactation	2	1.5	92.1	15.7	0.5	32.5	2.9		
Mild lactation	2	1.5	92.1	15.7	0.5	28.9	2.6		
Late lactation	3	1.2	111.1	18.9	0.30	27.1	2.4		
early pregnanc	4	1.2	148.1	25.2	0	0	0		
late pregnancy	1	1.2	37.0	6.3	0.30	9.0	0.8		
	12		480.5	81.7		97.5	8.8	14.5	10.3
Product	kg/year	Kg N from product/year			Total FI/yea	578.1			
wool	2	0.165							
milk	360	3.456							
lamb	8	0.576							
		4.2							

Assumptions:

- Ewe of 60 kg body weight
- 2 lambs produced per year, each with a body weight of 4 kg

- Lambs feed only 2 days from the ewe's milk
- 360 kg of milk produced per year in a 7 month lactation period (210 d)
- Milk contains 6% protein
- Yearly wool production of 2 kg, containing 33% keratin and 25% N in keratin.
- Maize containing 9% protein
- Alfalfa hay containing 17% protein.

14) Dairy goat

Stage of production	months	Kg feed DM/day	FI in Kg DM/year	CP (%)	kg protein/year	Kg N intake/year	Kg N excreted/year
Lactation	8	2.4	580.8	19.5	113.1	18.1	
dry period	2.5	1.1	80.9	10.5	8.5	1.4	
late gestation	1.5	1.2	52.7	13.7	7.2	1.2	
	12		714.3		128.8	20.6	16.5
Product	kg/year	Kg N from product/year					
milk	720	3.7					
kid	6	0.4					
		4.1					

Assumptions:

- Dairy goat of 60 kg body weight
- Production cycle consisting on a lactation period of 8 m, a maintenance (dry) period of 2.5 m and a late gestation period of 1.5 m.
- Yearly milk production of 720 kg in 240 d
- 1.5 Kits produced per year

15) Laying hen

Brown layer	weigt gain	Phase	Kg feed	% CP	Protein kg	N intake kg	kg N excreted/year
1,4-2kg	0.6	16-32 week	10.85	19.8	2.1	0.34	
		33-44 week	8.4	17.5	1.5	0.24	
		45-55 week	7.7	17.0	1.3	0.21	
		56-68 week	14.7	16.0	2.4	0.38	
			41.65			1.16	0.80
product	eggs/year	Kg egg	% protein	Kg N excreted in eggs per year			
egg	300	18.0	12.00	0.35			
product	kg/year	% protein chicken	Kg nitrogen in weight gain				
weight gain	0.6	17	0.016				

Assumptions:

- Brown layer hen HY line breed

- Hen's body weight at start (16 w of age) is 1.4 kg and increases to 2 kg at the end (68 w of age)
- Feed consumption is 0.08 kg in weeks 16 and 17; 0.095 kg in weeks 18 to 23; and 0.1 kg from week 24 onwards.
- Yearly egg production of 321 eggs (ITAVI, 2014)
- An egg weights 0.06 kg in average
- Chicken have a protein content of 20% body weight.
- N excreted in feathers is assumed to end up in the manure and in the environment.

16) Horse (adult, maintenance)

BW (kg)	FI (kg DM/year)	CP (% in feed)	Protein (kg/year)	N excreted (kg/year)
500	**3650**	10	365	**58.4**

Assumptions:

- Average mature horse of 500 kg body weight, in maintenance.
- Daily feed intake (DM) of 2% of body weight
- 10% of the daily feed intake (DM) is protein

17) Horse for fattening (to produce horse meat)

Phase	Daily diet	kg	FI/run	CP%	Prot intake/ run (kg)	N intake/ run (kg)	N retained/ run (kg)	N excretion/ run
I (6-8 m)	hay	2.00	120	12	14.4			
	bran	2.40	144	13	18.72			
	Soia meal	0.40	24	45	10.8			
	Maize meal	1.2	72	9.5	6.8			
		6	360		50.8	8.1	1.6	6.5
II (8-11 m)	hay	2.00	180	12	21.6			
	bran	3.15	284	13	36.855			
	Soia meal	0.45	41	45	18.225			
	Maize meal	1.4	126	9.5	12.0			
		7	630		88.7	14.2	2.8	11.3
III (11-13 m)	hay	3.50	210	12	25.2			
	bran	4.50	270	13	35.1			
	Soia meal	0.50	30	45	13.5			
	Maize meal	1.5	90	9.5	8.6			
		10	600		82.4	13.2	2.6	10.5
	All three phases							
	FI one run (7 m)		1590		221.8	35.5	7.1	28.4
	FI total (1.5 run)		**2385**					**42.6**

Assumptions:

- Heavy (draft) horse that will reach an adult weight of 700-800 kg (eg. Belgian Ardennes, Breton, Comtois breeds)
- Production cycle of 7 months, starting at weaning (6 m of age) with a body weight of 270 kg and finishing at 13 m of age with a body weight of 480 kg.
- Daily weight gain is 1 kg
- Feed to gain ratio is 7.5 kg feed/kg body weight

- Number of production cycles/year is 1.5 (limited by the seasonality of the oestrus)
- Feed contains 14% crude protein along the whole production cycle
- N retained from ingested is 20%